A Chemical Study of the Placental Hormone

DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF PURE SCIENCE, COLUMBIA UNIVERSITY

By

PAUL MILLER GIESY, M. A.

NEW YORK CITY

1920

781
72G
CLASSED

THE LOGES-WIENER COMPANY
PRINTERS—PUBLISHERS
NEWARK, N. J.

STANFORD UNIVERSITY LIBRARIES

A Chemical Study of the Placental Hormone

DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF PURE SCIENCE, COLUMBIA UNIVERSITY

p.72, line 21: For "Stock II", read "Stock I".

By

PAUL MILLER GIESY, M. A.

NEW YORK CITY

1920

THE LOGES-WIENER COMPANY
PRINTERS—PUBLISHERS
NEWARK, N. J.

TO MY WIFE

ACKNOWLEDGMENT

The author desires to express his indebtedness to Dr. Robert T. Frank, who suggested this problem, and with whose coöperation the work was carried on for a year. He is also grateful to Mr. O. I. Lee for his helpful advice. The assistance of the Staffs of the Bacteriological Laboratory of St. Luke's Hospital and of the Crocker Laboratory in those portions of the work requiring bacteriological and physiological technic has been deeply appreciated. The author's sincerest gratitude is due to Professor Francis Carter Wood, whose guidance of the work in all its phases has been invaluable.

ABSTRACT OF DISSERTATION

1. What was attempted?

2. In how far were the attempts successful?

3. What contribution actually new to the science of chemistry has been made?

1. The attempt was made (*a*) to work out a simple method for the extraction and concentration of the active substance of the placenta, in order to make this available for physiological work; (*b*) to determine whether the physiological test for the presence of the substance could be developed into a method for its quantitative determination; (*c*) to determine the solubility of the substance in various solvents; (*d*) to study the partition of the substance between various pairs of immiscible solvents; (*e*) to determine whether the substance dialysed in various solvents; (*f*) to study the effect of various conditions and reagents upon the activity of the substance; (*g*) to determine something of its chemical nature.

2. (*a*) Definite conditions have been worked out for a simple and reliable method of extracting and concentrating the active substance.

(*b*) It has been found that the variation in susceptibility of the test animals is too great to permit the physiological test to give results which are better than roughly quantitative.

(*c*) The substance has been found to be soluble, under definitely described conditions, in benzene, chloroform, carbon tetrachloride, absolute and 95% alcohol, ether, ethyl acetate, carbon disulfide, pyridine, acetone, acetyl chloride, and hot acetic anhydride. It was somewhat soluble in 50% alcohol and glacial acetic acid. Under certain conditions, it was soluble in water and benzin.

(*d*) With water and benzene, no active substance was found in the aqueous layer, while the benzene layer was active. With water and benzin, the distribution depended on the other substances present. With benzin and 75% alcohol, most of the active substance was present in the alcoholic phase.

(*e*) With rubber membranes, the substance dialysed in acetone, benzene, ether, and chloroform solution, and dialysed slightly in benzin and

carbon tetrachloride solution. With collodion membranes, the substance dialysed in 62½% and 75% alcohol solution, but not in 50% alcohol solution.

(*f*) The activity was not destroyed by heat, except in the presence of air. Alcoholic solutions lost activity on standing; benzene solutions did not. The activity was slowly destroyed by acids, alkalies, and bromine, and rapidly by nitrous acid and cold alkaline permanganate. It was not destroyed by acetyl chloride, boiling acetic anhydride, zinc and glacial acetic acid, aqueous sodium carbonate, sodium bisulfite, cadmium chloride, fuller's earth, phenylhydrazine, hydrocyanic acid, hydroxylamine, or semicarbazide.

(*g*) A concentrated preparation of the substance contained neither nitrogen, sulfur, nor halogens. The tentative conclusion has been reached that the active substance is probably an unsaturated compound of ester-like nature, containing neither the carbonyl, carboxyl, amino, imino, nor hydroxyl grouping.

3. Practically the whole of the above is pioneer work. It has been previously stated that the active substance contains only carbon, hydrogen, and oxygen, and that it is soluble in alcohol, ether, petroleum ether, acetone, and benzene, insoluble in water. With these exceptions, all the results given under 2. above are contributions actually new to the science of chemistry, and were obtained in this investigation.

A CHEMICAL STUDY OF THE PLACENTAL HORMONE

CONTENTS

I. Introduction.

II. Discussion of Results.

A. Preliminary Work. B. Preparation of Stock Materials. C. Physiological Tests. D. Solubility Experiments. E. Partition Experiments. F. Dialysis Experiments. G. Effect of Conditions and Reagents. H. Progress Toward the Preparation of the Pure Active Substance.

III. Experimental. (Same subdivisions as II.)

IV. Summary.

I. INTRODUCTION

The growth of the uterus and mammary glands during pregnancy has for a long time been of interest to physiologists. In 1905 Halban[1], on clinical and physiological grounds, proposed the theory that their growth was due to some substance secreted by the placenta. The early attempts to verify this were inconclusive. Claypon and Starling[2] injected placental press-juice into female rabbits. The mammary glands were unaltered; the uteri were congested. Frank[3] injected emulsions of rabbit placentas into female rabbits, but noticed no change in the genital organs. Biedl and Königstein[4] implanted placentas and injected placental extracts, but no breast or uterus growth resulted. Frank and Unger[5] repeated the experiments of Claypon and Starling, but failed to obtain the same results. This they attributed to the cyclical variation of normal mammary glands, connected with rut. Aschner and Grigoriu[6] reported that the injection of placenta pulp or its aqueous extract caused a true milk secretion and hypertrophy of the vascular glands. They stated that the substance producing these effects is soluble in water and is destroyed by alcohol and heat. Niklas[7] found that lacteal secretion of short duration, preceded by hyperplasia of the mammary glands, was produced by the action of placental substance on both virginal animals and those which had borne young. Aschner[8] found that injection of placental extract into guinea-pigs produced rut-like symptoms; hyperemia occurred even in castrated animals, though to a less extent than in normal ones. Okintschitz[9] found that physiological salt solution extracts of chorion from two to three month human embryos prevented the atrophy of the uteri of rabbits after castration.

The cause of this growth of the uterus and mammary glands has also been sought in the ovary and corpus luteum. Experiments similar to the above, using ovarian tissue and extracts, were made by Claypon and Starling[1], Bucura[10], Adler[11], Schickele[12], Sack[13], Aschner and Grigoriu[6], Aschner[8], Okintschitz[9], Ott and Scott[14], an anonymous author[15], and Ssoloviev[16]. All except Aschner and Grigoriu, Okintschitz, and Ott and Scott observed rut-like symptoms. Ott and Scott and Ssoloviev reported enlargement of the mammary glands.

The reason for the failure to obtain positive results with aqueous extracts became evident from the work of Fellner and Iscovesco, which showed the fat-like solubility of the substance. Fellner[17] extracted minced placentas with alcohol and ether, evaporated the extract, and took up the residue in physiological salt solution. Two weeks' injection of this emulsion into female rabbits produced hypertrophy and hyperplasia of the uterus, growth of the mammae, and the formation of acini. Injection into males produced breast growth. Parodi[18] confirmed this. In a later, more extended paper[19], Fellner stated that the substance was soluble in alcohol, ether, and acetone. He extracted minced placentas with boiling alcohol, dried the residue, and again extracted with petroleum ether. The combined extracts were evaporated, and the residue was treated with absolute alcohol and ether. The filtered solution was evaporated, and the residue taken up in physiological salt solution. The emulsion was sterilized before injection. Aqueous extracts of washed placentas were less active than saline extracts, and these again less powerful than alcohol or acetone extracts. Extracts of ovaries containing corpora lutea were active; extracts of ovaries without corpora lutea were inactive. Iscovesco[20], applying to ovary lipoids a method previously used[21] for the investigation of kidney lipoids, obtained a fraction which he injected subcutaneously into female rabbits. After 18 injections of 30 mg. each, on autopsy the uterus was found enlarged to about three times its normal weight, and very congested. In other cases where the injections were continued several months longer the uteri were found to be enlarged to from five to seven times normal size. Another[22] lipoid fraction from the ovary produced similar effects. Seitz, Wintz, and Fingerhut[23] isolated a lecith-albumin "lipamin" from cow corpus luteum by extracting with alcohol, adding an excess of chloroform, removing the aqueous layer, and repeatedly extracting the alcohol-chloroform solution with water. The aqueous solutions were evaporated. The residue, after washing with ether, was dissolved in physiological salt solution and injected into rabbits. The uterus and ovaries increased in size and became hyperemic and soft.

The most important contributions in this field have been made by

Edmund Herrmann. In 1913[24] he reported the isolation from corpus luteum of an unsaturated phosphatide of nitrogen-phosphorus ratio 5:2. Seven daily injections of 0.01 g. each into rabbits caused hyperemia of all the genitals, swelling of the uterine mucosa, and secretion by the uterine and breast glands. Several weeks' injection caused considerable enlargement of all the genitals and the breast glands. The uterus thickened six- to eight-fold, with hypertrophy of the muscular as well as of the mucous coat. In the breast there was hypertrophy and hyperplasia of the acini. The ovaries were enlarged, with many ripening follicles. The sexual cycle was reduced from four weeks to two. The same phosphatide was said to be present in the placenta.

In a later communication[25] Herrmann gave the method of preparation of this phosphatide. Ground corpora lutea were extracted with acetone, and the dissolved material was fractionated with absolute alcohol and acetone. The same phosphatide was obtained from ovaries containing no corpora lutea. He stated that the pure active substance was prepared in the following manner: The minced organs, dried in vacuo at 37°, were extracted with ether in a Soxhlet apparatus for 48 hours. The ether was evaporated and the residue treated with three to four volumes of acetone. The activity of the precipitate obtained thereby was due to adsorption. By resolution in absolute alcohol or ether and reprecipitation with acetone it was finally obtained inactive. The mother liquor was evaporated and cooled, cholesterol separating. Cholesterol esters and neutral fats were separated by cooling the ethyl and methyl alcohol solutions respectively. Finally the material was distilled in an "absolute" vacuum; the middle fraction, distilling at 193° (uncorrected) at 0.06 mm. pressure, contained the active principle. The thick liquid was rubbed with 95% alcohol. After separation from the cholesterol esters, the solution was evaporated and again distilled in an "absolute" vacuum. The active principle Herrmann described as a yellow, slightly fluorescent oil, solidifying on cooling, otherwise a thick liquid. It gave a pronounced cholesterol reaction, and turned brown in the air by oxygen-absorption. It contained only carbon, hydrogen, and oxygen, and analysed by combustion C 81.33-81.62%, H 11.32-11.49%. "It is a cholesterol derivative, is soluble in alcohol, ether, petroleum ether, acetone, and benzene, insoluble in water." It was stated that the placenta contained the same substance. The physiological effects of injection of the substance into rabbits are given in great detail. Herrmann's process has been patented in Austria[26], Great Britain[27], and the United States[28].

Frank and Rosenbloom[29] extracted the active principle from placentas with alcohol, ether, acetone, and chloroform. They stated that extracts

containing all the fat-soluble substances were by far the most potent. Extracts of corpora lutea from pregnant animals were almost as potent as placental extracts, while those of corpora lutea from non-pregnant animals were inert. Degreasing the corpora lutea removed the active substance. The effect of these active extracts upon the mammary gland was apparently slight. In a later paper Frank[30] reported a marked hyperplasia of the mammary glands of rabbits; rats and guinea pigs were less susceptible. His potent crude extract was obtained by continuous extraction of placentas with alcohol. It was a dark, semi-liquid mixture which resisted high degrees of heat (350°), strong acids or *alkalies and saponification without loss of potency. It either oxidized or polymerized on standing, gradually losing its activity.* It was fully soluble in 95% alcohol, partially soluble in chloroform, ether, oils and benzin, the active fraction at times following the benzin-insoluble portions. The activity persisted after removal of cholesterol and indole. The italicized statements were based on work done in coöperation with the present author.

Lee and Frank[31] found that the activity was not removed from preparations by treatment with phosphotungstic, phosphomolybdic, or picric acids. The activity was removed by treatment with alcoholic mercurous nitrate, chromic anhydride in ether, stannic chloride in ether, and selenium oxychloride in anhydrous ether. The activity was not recovered by treating the alcoholic mercurous nitrate precipitate with hydrogen sulfide.

Cova[32] extracted placentas with hot alcohol and evaporated the extract. The portion soluble in ether and petroleum ether was emulsified in physiological salt solution and injected into rabbits and guinea pigs. Uterus and breast growth resulted. Gentili and Binaghi[33] extracted the dried maternal portion of cow placenta with alcohol, and evaporated the extract to dryness. The acetone-insoluble phosphatide they injected into rabbits; the mammary glands were excited to secretion, while the development of uterus, ovaries, and tubes was diminished.

At the commencement of the present study, then, the following was known:

(1) A substance exists in the placenta which, injected into female animals, stimulates uterus and mammary gland growth.

(2) The substance can be extracted from the minced fresh organs by alcohol and acetone, and, in relatively very small amounts, by water or physiological salt solution. From the minced, dried organs it can be extracted with alcohol, ether, acetone, and chloroform. No definite conditions were given for the extraction of fresh organs.

(3) Under certain conditions, which are nowhere definitely stated, the

substance is soluble in alcohol, ether, acetone, benzin, benzene, chloroform, and methyl alcohol, and insoluble in benzin and water.

(4) The substance may be recovered unchanged, *in part at least*, after distillation or treatment with strong acids or alkalies, phosphotungstic, phosphomolybdic, or picric acids.

(5) The activity is removed or destroyed by alcoholic mercurous nitrate, or ethereal chromic anhydride, stannic chloride, or selenium oxychloride.

The present study was begun at the suggestion of Dr. Robert T. Frank, and for a year carried on with his coöperation. It was interrupted by the military service of Dr. Frank and the present author. The illness of Dr. Frank has prevented his resuming work on the problem.

New methods have been developed for the extraction of the active material from placentas, of which the best is that of extracting the substance in 60% alcohol solution, evaporating the alcohol, and extracting the emulsion with benzene. The active substance has been further concentrated by dialysing it in acetone solution through rubber, and then in 62½% alcohol solution through collodion membranes. (See, for details, Experimental, B, *Preparation of Stock Materials.*) As far as the author is aware, this is the first time this method has been employed in the purification of physiologically active lipoids.

On treatment of the stock materials with solvents, the substance has been shown to dissolve in benzene, chloroform, carbon tetrachloride, absolute, 95%, and 50% alcohol, ether, ethyl acetate, carbon disulfide, pyridine, acetic anhydride, and acetone, and to dissolve somewhat in glacial acetic acid. With "Stock I," it was not dissolved by benzin, but was by water, though the water-insoluble fraction was more active. With "Stock II," the benzin solution was somewhat active. Previous workers, using materials of indefinite preparation and composition, have made unjustifiable statements as to solubility, when this, with lipoids, depends often on what other substances are present. In this work, definite conditions are given for the preparation of materials, and so the solubility determinations have some real meaning.

From the results of partition experiments with benzene and water, the aqueous phase was found to be inactive, and the benzene, active; with benzin and water, varying results were obtained, depending on the other substances present; with benzin and 50% or 75% alcohol, the bulk of the active substance was in the alcoholic layer.

The substance dialysed through rubber membranes in acetone, benzene, ether, and chloroform solution, and dialysed slightly in benzin and

carbon tetrachloride solution; through collodion membranes, it dialysed in 75% and 62½% alcohol, but not in 50%.

No destruction of activity was found on prolonged heating, either in the dry state, in alcoholic solution, or in aqueous emulsion, provided air was excluded. Heating in the air destroyed the activity. One preparation, after remaining in the dry state for two and a half years in a corked flask, was still active. Activity was not lost on standing in benzene solution, but it was in alcoholic solution. In a cotton-stoppered bottle, the aqueous emulsion lost its activity largely in three weeks.

All attempts to prepare derivatives which could be purified easily were unsuccessful. Active material on treatment with the ordinary reagents for hydroxyl, carbonyl, and carboxyl groups did not lose its activity; it was therefore concluded that the hormone did not contain these groups. Active material did not lose its activity on reduction, but the activity was destroyed by oxidizing agents, bromine, acids, and alkalies. These results lead to the tentative conclusion that the substance is unsaturated, and of ester-like nature. "Stock III," the most concentrated preparation obtained, gave no test for nitrogen, phosphorus, or halogens. Since this agrees with the observation of Herrmann on his "pure substance," prepared in entirely different fashion, it is probable that the destruction of the activity which nitrous acid causes is due to oxidation, and not to deamination. The failure of acetylation to destroy the activity confirms this.

II. DISCUSSION OF RESULTS

A. Preliminary Work

The first experiments were made upon "Stock A," the preparation of which is described in No. 1. Later, "Stock B" was used, the preparation of which is described in No. 11. Still later, cold alcoholic extracts of fresh placentas were employed as starting materials.

Herrmann's work was not repeated for two reasons. First, such meager details are given of his chemical work that it would be impossible for anyone to be sure of duplicating his exact conditions. Second, the facilities were not at hand for drying placentas in sufficient quantity for the purpose. In No. 2, an attempt was made to apply his method of fractionation to "Stock A," but the results were not promising. The only conclusions to be drawn were that, in the case of this particular mixture, the active substance was soluble in acetone, ether, and ethyl and methyl alcohols, and that it could be distilled unchanged, at least in part, under low pressures, at temperatures of 160° to 357°.

Samples of "Stock B" after treatment with alcoholic sulfuric acid and alcoholic potash were still active. Because of this, and similar results obtained by Lee and Frank with acid treatment of active material, it was concluded that the active substance was unaffected by acids or alkalies; this conclusion, however, has been shown by later work to have been erroneous. Apparently none of the active material was combined with the alkali, since on acidifying the extracted alkaline solution and shaking out with ether, the extracted material was inactive.

Some active material was obtained by extracting fresh ground placentas with water, but the yield was very low. After drying the extracted pulp, it was found still to contain active material.

The long-continued heating in the still of the Soxhlet apparatus during the preparation of "Stock A" resulted in the change of the labile lipoids to such an extent that the final material, and the "Stock B" prepared from it, were black, tarry masses, extremely difficult to work with. To avoid this, the press-juice from the first alcoholic extraction of fresh placentas was boiled down over a free flame, and then worked up as for "Stock B." The product was of a light brown color, and evidently much less affected by the heating. Later work showed that vacuum evaporation gave still better results.

The final product of No. 34 tested +2½; that of No. 36, prepared similarly, and injected in about one-third the concentration of the other, tested +3. This led to doubt as to whether the test-number on Dr. Frank's scale was proportional to the amount of active substance in the preparation. Accordingly, several preparations were tested in varying dilutions. In No. 37, a 12.5% solution tested less than 6.3% and 2.5% solutions. In No. 38, 5%, 2.5%, and 1.25% solutions all tested the same. In No. 39, 2.5% and 0.5% solutions tested +2, 0.1%, +1¾, and 0.02% inactive. These results showed the impossibility of drawing any quantitative conclusions from the results of single physiological tests.

Since the second alcoholic extract of fresh placentas contained so much more solid material than the first extract, a good deal of work was done with it. It eventually became evident, however, that it contained relatively much less of the active material than did the first extract, much more inert material being extracted by the stronger alcohol.

From other portions of this preliminary work, the following appeared to be true: the active substance in these mixtures is soluble in ethyl and methyl alcohol and mixtures of these with ether, in ether, ethyl acetate, acetone, 50% alcohol, and 50% acetone, slightly soluble in 25% alcohol, and insoluble in water and 78° benzin; it dialyses through collodion membranes in 95% alcohol solution. The ether extract of dried

placentas was only slightly active, much less so than the material prepared by applying the method of preparation of "Stock B" to the first alcoholic extract of placentas. While the active substance is insoluble in water, the aqueous extract of fresh placentas contains a small amount of the substance in colloidal solution. Active material, after standing exposed to the air for several months, loses its activity. The avoidance of temperatures as high as that of the steam bath gives a much better product. The activity is not destroyed by cold acetyl chloride, and so the substance probably contains no amino or hydroxyl groups.

B. Preparation of Stock Materials

The methods of both Herrmann and Lee have disadvantages. Herrmann's method requires a vacuum drier and a large scale Soxhlet extractor, which are not available in the ordinary laboratory. The material obtained by the ether extraction is of relatively low activity, and must be concentrated by a time-consuming process of fractionation by solvents, for which only indefinite conditions are given. The final fractional distillations at 0.06 mm. pressure require apparatus not usually available.

Lee's procedure also requires special apparatus, in the shape of a large scale Soxhlet extractor. The long-continued extraction with alcohol extracts inactive lipoids, and probably also some protein, since the extract on distillation with superheated steam gives a distillate which smells strongly of ammonia and amines. As the result of overheating in the still of the extraction apparatus, the product obtained on evaporation of the alcohol resembles soft tar in color and consistency.

The method for "Stock II" developed in the present work possesses several advantages over the two previous ones. Aside from a centrifuge and a small mechanical vacuum pump, only ordinary flasks, etc., are required. If, as is possible, the quality of the product would not be injured by heating to 50° or 60° during the evaporations, the mechanical vacuum pump could be dispensed with, and only water pumps used. The material obtained ("Stock II") is much more active than the original extracts of either Herrmann or Lee. For physiological experiments it should require no further concentration, but if maximum obtainable effects are desired, it can be concentrated by the "Stock III" procedure, which requires no special apparatus.

It should, however, be said that while sufficient yields for physiological experiments can be obtained by working with the apparatus later described, for chemical work having in view the purification of the substance, larger scale preparation is necessary. The author believes that no further

progress can be made toward the preparation of the chemically pure hormone unless work is done on a large enough scale to require a vacuum evaporator and large centrifuge (holding liter bottles). The small scale apparatus will handle the extract from about 30 lbs. of placentas, giving about 3 g. of "Stock III," per week. While, by the use of a higher temperature in evaporation, this could be doubled, it is evident that the yield would be too small for, say, fractional distillation experiments.

Herrmann's method was essentially that of extracting all the lipoids, and afterwards fractionating them. The methods described here were based on the opposite idea of trying to extract as little inert material as possible, while getting all the active material. For the first extraction of the minced placentas, sufficient alcohol was used to give a final extract of 55-60% alcohol; practically all the active material is extracted by alcohol of this concentration. A final concentration of 70% extracts additional inert material. A lower concentration prolongs the evaporation of the extract greatly.

The preliminary work had shown the advantage of vacuum evaporations in preventing heat changes of the labile lipoids present. Consequently, all evaporations were conducted under reduced pressure, at temperatures not exceeding 40°, except in the evaporation of acetone in the preparation of "Stock III," where the bulk of the solvent was boiled off on the steam bath, and the evaporation was finished under reduced pressure.

The use of acetone instead of alcohol in the first extraction was tried, but appeared to possess no particular advantage. Alcohol was therefore used on account of its lower cost.

In the preliminary work, different materials prepared from alcoholic press-juice from fresh placentas by the "Stock B" procedure showed varying activities on test. It was thought that this might be due to variations in the composition of the alcohol-ether mixtures used. Consequently, in the preparation of "Stock I," ether was eliminated entirely in the effort to secure exactly reproducible conditions. (It had been planned to study the effect of the addition of ether later, but when the solubility and partition experiments showed the availability of benzene as an extraction agent, further work with ether was dropped.) The filtered alcoholic extract of fresh placentas was evaporated to dryness, and the residue was emulsified with water. Five volumes of 95% alcohol were then added; after settling over night, the solution was filtered. The residue after evaporation has been called "Stock I". It was found that the use of less than five volumes of alcohol to one volume of water in the preparation of the final solution did not give complete precipitation of alcohol-insoluble

matter. The solution obtained also foamed very badly during evaporation. It was later found that the use of ten volumes of alcohol to one of water decreased somewhat the foaming during evaporation, without changing appreciably the amount of precipitate.

"Stock II" was prepared by evaporating filtered alcoholic extract of fresh placentas to about one-tenth its original volume, extracting with benzene, and centrifuging and evaporating the extract. The best method developed for the further concentration of the active material was the dialysis of "Stock II" in acetone through thin rubber, and then in 62½% alcohol through collodion. By this procedure, lecithin and other colloids are removed, together with the bulk of the cholesterol.

That the active material was actually concentrated by this procedure may be inferred from the fact that the yield of "Stock III" was at most only one-quarter of the "Stock II" used. It is more definitely shown by comparison of the reactions obtained on test with "Stock II" (Nos. 153 and 154) with those given by "Stock III" (Fig. 5, Nos. 61-69).

During the handling of the fresh placentas, precautions were taken against infection by Treponema pallidum or any other organisms which might have been present in the organs. But after the material had stood over night with alcohol, cultures prepared from it gave no growth in three days; consequently no special care was taken from then on. The use of acetone in the first extraction also gave sterile material.

From 10 lbs. of fresh, ground placentas were to be obtained, on the average, 42 g. of "Stock I," 5½ g. of "Stock II," and 1 g. of "Stock III". With the available facilities for evaporation, the yield of "Stock III" was limited to about 3 g. per week.

Autolysis of the organs preceding the first extraction resulted in a much greater amount of extract insoluble in the final alcoholic liquor in the preparation of "Stock I". From this, it seemed possible that there might be degradation-products in the final material also, which would make subsequent purification still more difficult. Consequently, every effort was made to prevent any change in the organs before the extraction.

C. Physiological Tests

At the present time no method exists for determining the presence or amount of the active substance in a preparation, other than the observation of its physiological effect. The tests used by previous investigators have differed greatly, so that it is impossible to compare the concentrations of their various preparations from the results of the tests. Herrmann[25], using young female rabbits of about 800 g. weight, injected sterile

olive-oil emulsions every other day; the animals received 30 mg. of "pure substance" at each of the first two injections, and 60 mg. at later ones. Hermann described the appearance of the genitals after two, three, four, and five injections, but apparently made no attempt to study the effect of varying the amount of "pure substance" injected.

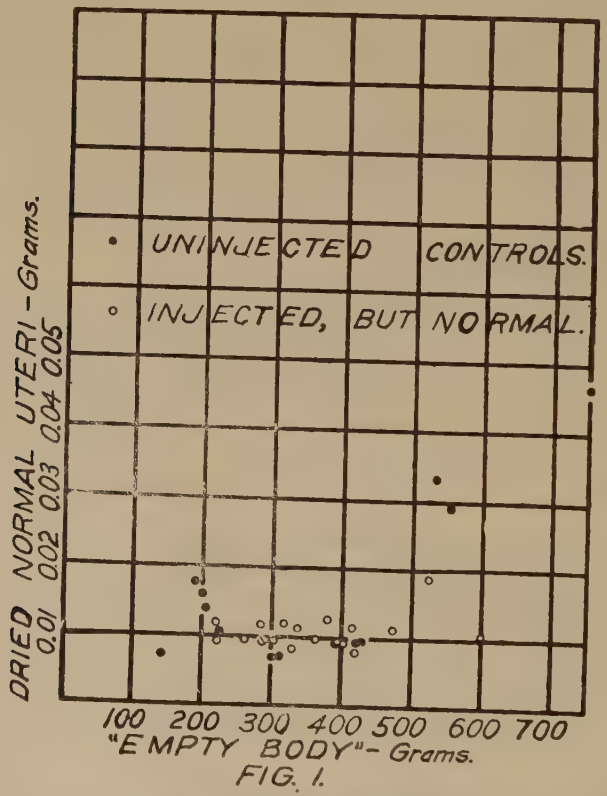
To Frank[30] belongs the credit of being the first to use the physiological effect of the substance as an indication of the amount of it present in a preparation. He injected four portions of the substance to be tested at two-day intervals, each injection containing 25 mg. The animal was killed two days after the fourth injection, and the condition of the uterus reported as "plus one," "plus three," etc. Unfortunately, this method is useless in comparing his preparations with any other person's.

In the present work, in deciding on the method of test, an endeavor was made to reduce the time required for the test as far as possible; consequently only a single injection was used. Three animals were injected with the same amount of material, and killed after two, four, and eight days. The greatest effect was observed with the four-day animal, consequently this period was chosen.

Later on in the work it became evident that the number of experiments on which the period of four days was based was too small to give accurate results. It seems quite probable that with high concentrations of active material maximum growth would not be reached until after a longer period. Rather than change the procedure at that point, however, it seemed advisable to continue using the four-day period in order to have results comparable with the earlier ones.

A single injection, usually containing twenty-five milligrams of material, emulsified in mucilage of tragacanth, was used, either prepared aseptically, or sterilized after preparation. In pharmacological work, ordinarily dosages are used which are a definite fraction of the weight of the animal. This is logical when the concentration of the drug in the body is the important thing. But in this work, the important factor would seem to be the amount of active material which reaches the uterus. Since all of the injected material absorbed into the circulation will probably do this eventually, in this problem a constant amount of injected material, regardless of the weight of the animal, seems more logical. In any case, the variation introduced by this factor would be less than that caused by ununiform absorption, or varying susceptibility of the animals. Where the effect of a reagent on the active substance was being studied, the final product was diluted to the same volume as would have given a concentration of 2.5%, had the original material been diluted to that volume; one cubic centimeter was injected. Four days after injection, the

animal was killed, and the uterus and vagina were dissected out. Since uterus growth was being studied, the most natural objective standard to use in measuring it seemed to be the weight of the organ. To eliminate as far as possible any effect of edema on the weight, the organ was dried over sulfuric acid in vacuo to constant weight. As controls, a series of uninjected animals were killed and the uteri dried and weighed. Fig. 1 shows the weight of dried normal uteri as a function of "empty body"


UNINJECTED CONTROLS.
INJECTED, BUT NORMAL.
DRIED NORMAL UTERI - Grams.
0.01 0.02 0.03 0.04 0.05
100 200 300 400 500 600 700
"EMPTY BODY" - Grams.
FIG. 1.

Normal uterus weight as a function of "empty body" weight.

weight. The "injected, but normal" points in the figure are the weights of the uteri of animals which were injected, in tests, with materials which had no effect on the uterus. To eliminate the effect of varying stomach and intestinal contents, the stomach and intestines were removed from each

carcass and the remainder, called the "empty body," was weighed. Fig. 2, showing the weight of live animals as a function of "empty body" weight, gives an idea of the variation due to varying contents of the digestive tract. With twelve animals, the digestive tracts after removal

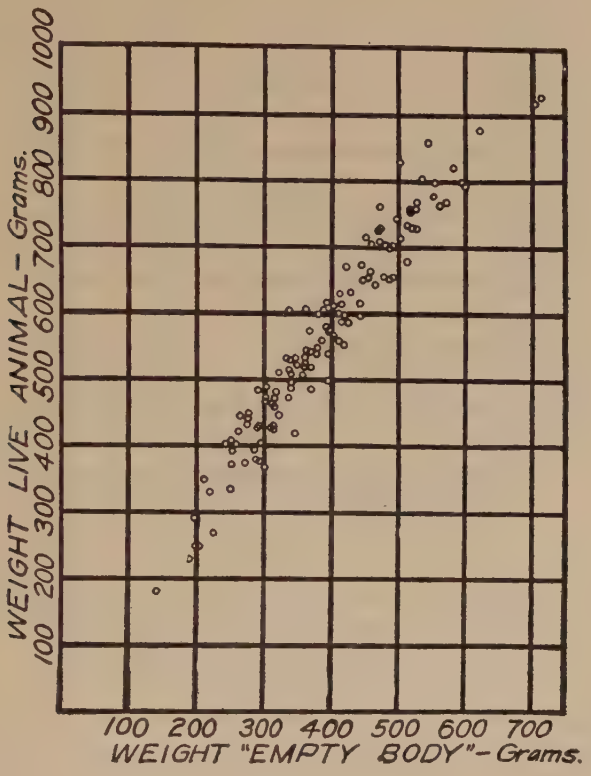
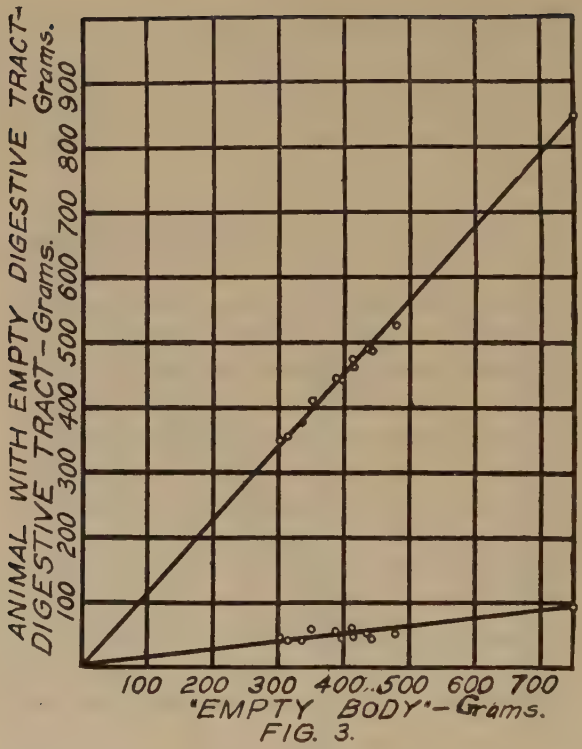


Fig. 2.

Weight of live animal as a function of "empty body" weight.

were cleaned, washed, dried between towels, and weighed; the weight was found to be nearly a linear function of the "empty body" weight, the variation being much less than the normal variation in physiological effect, so this time-consuming process was no longer employed. Fig. 3 shows the weight of digestive tracts and of the animals with empty digestive tracts as a function of "empty body" weight.


ANIMAL WITH EMPTY DIGESTIVE TRACT-Grams.
DIGESTIVE TRACT-Grams.
100 200 300 400 500 600 700 800 900
100 200 300 400 500 600 700
"EMPTY BODY"-Grams.
FIG. 3.

Weight of digestive tract and animal with empty digestive tract as functions of "empty body" weight.

Following the procedures of Herrmann and of Frank, young female rabbits were used as the test animals The ages varied from six to ten weeks, the weight never being over 900 g. As Herrmann has pointed out, rut does not occur until the animals are five months old; consequently this source of variation was eliminated. The series of normal uteri showed that the weight varied least for empty body weights of between 225 and 450 g. All animals weighing from 380 to 600 g. fell within these limits. Such animals, however, were not always obtainable, so that part of the work had to be done with larger or smaller animals.

The preliminary work had showed that, even after long experience, the visual estimation of the effect of various preparations could lead to the rating of one material as more active than another which actually con-

tained four times as much active substance. It was hoped that the weighing of the uteri might lead to more concordant results, but it did not seem to. In the first series of experiments on reaction as a function of the weight of active material injected, the uteri of the rabbits which received the 4.76% and 9.5% emulsions were larger than that of the rabbit which received the 19% emulsion. In the second series, the uterus of the animal which was injected with 10% emulsion was smaller than that of the one injected with 5% emulsion. Fig. 4 gives graphically the re-

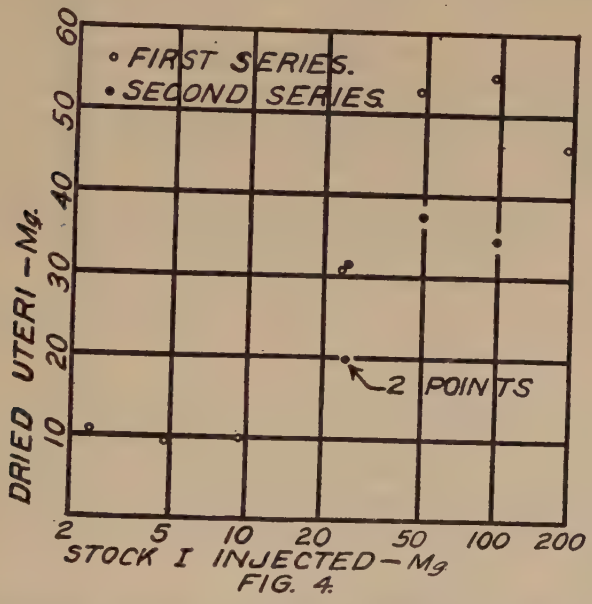


FIG. 4.

Weight of dried uterus as a function of weight of "Stock I" injected.

sults of the first (Nos. 50-56) and second (Nos. 57-60) series. The variation in reaction is also shown in the cases where several different rabbits were injected with the same emulsion (Nos. 59, 115, 150, and 191). The results indicate that only by the use of a number of animals for each test can the method yield even approximately quantitative results. It is also evident from the experiments of the "Stock III" series, where 0.25 cc. of 1.44% emulsion gave no reaction, and 0.10 cc. a slight one, that the minimum dosage for noticeable effect varies quite as much. Fig. 5 shows the results of the third series (Nos. 61-69), using "Stock III".

The observed physiological effect of the active substance is that of an enlargement of the uterus, vagina, and tubes. The ovaries, to the eye,

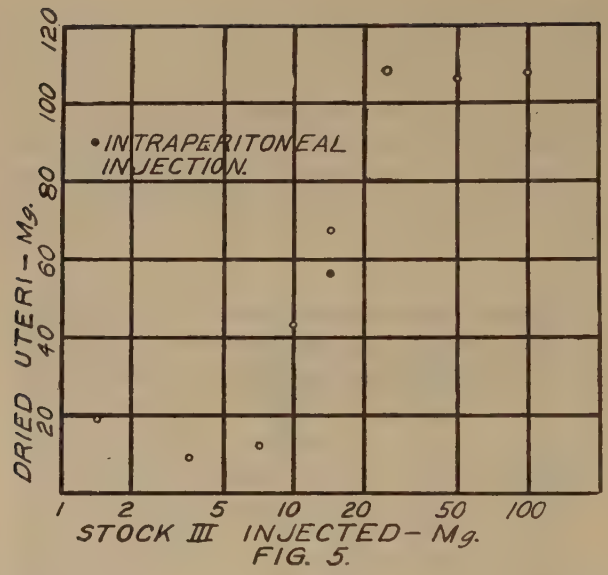


FIG. 5.

Weight of dried uterus as a function of weight of "Stock III" injected.

were unaffected. No observations were made as to the effect on the breasts. The enlargement was almost always accompanied by a congestion of the vessels of the broad ligament and the uterus. Occasionally, however, there was growth without any congestion. In cases where there was but little effect from the injected material, the hypertrophy and congestion were frequently confined to a portion of the uterus. With such materials, activity could be recognized visually by the localized congestion and enlargement when it could not be proved by increase of weight. In instances where a very active material was injected, there was congestion of and secretion of mucus by the external genitals.

How, then, it will be asked, can conclusions be drawn from tests made by a method which has been demonstrated not to be quantitative? First, by the use of the method as a purely qualitative test for the active substance. "Stock II" always produces a strong reaction, causing growth of the uterus to from four to ten times its normal weight. After treatment of this with nitrous acid (Nos. 184 and 185) or alkaline permanganate (No. 180), the resulting material, diluted to the volume to which the original "Stock II" would have been diluted for test, gave no reaction whatever. Obviously, the active material must have been destroyed, though not necessarily completely. Similarly, when "Stock I" was shaken

with water and benzene, and the phases were evaporated separately (Nos. 105 and 106), the benzene-soluble fraction showed activity, while the water-soluble one did not. Obviously, here there was a concentration of the active substance in the benzene solution. It cannot be said from the result of the test that *none* of the active substance was in the aqueous layer, although this can be deduced from later work showing that the active substance forms true solutions in benzene and colloidal ones in water.

Second, by use of the test as one in which the effect produced on the uterus by one material is always greater than that produced by another if there is sufficient difference in the concentrations of the active substance in the two materials. In general, the effects of injected materials upon the uterus may be grouped into four classes, each of which corresponds to a certain range of concentration of the active substance: (1) no noticeable effect; (2) slight effect: either localized congestion and hypertrophy, or slight general enlargement; (3) moderate effect: the uterus is congested and weighs (dried) from 30 to 100 mg., approximately; within this range is the greatest variation of effect; (4) extreme effect: the uterus grows at its maximum rate; no greater increase is obtained by increasing the dosage; the three uppermost points in "Fig. 5" lie in this range. These ranges,

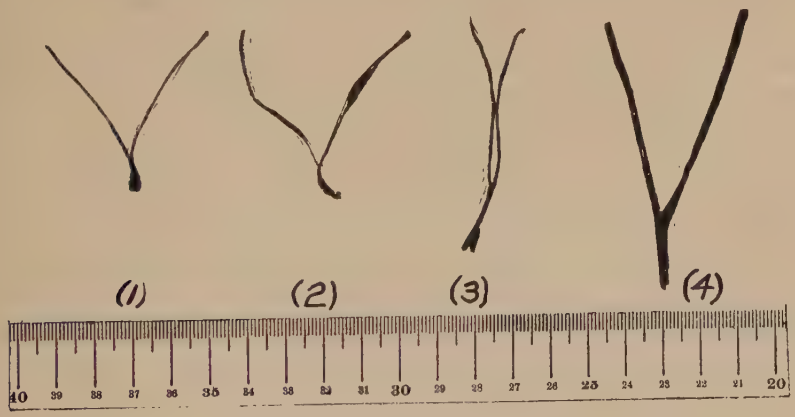


FIG. 6.

Uteri showing typical reactions in various ranges of effect—

(1) No effect;
(2) Slight effect, localized reaction;
(3) Slight effect, diffuse reaction;
(4) Maximum effect.

The scale in the figure is one of centimeters.

of course, overlap more or less. The third series of experiments upon reaction as a function of dosage (Fig. 5) shows an overlapping of the two lowest ranges (Nos. 66-68). "Stock II" usually gives reactions which lie in the upper part of the range of moderate effect, but occasionally a reaction is obtained in the range of extreme effect. Consequently, if "Stock II," after some treatment whose effect is to be ascertained, is tested, and gives a reaction in the lower part of the range of moderate effect, no conclusion can be drawn from the single test, other than the one that some active substance is still present. Such a case is that of the soy-bean lipase hydrolysis (No. 171). But if the reaction is in the range of slight effect, it is extremely probable that the material contains much less active substance than did the original "Stock II".

Only two instances have been noted which furnish possible exceptions to these statements. In the benzene dialysis (Nos. 132-135), the first trial gave a reaction in the range of slight effect, the last two, reactions in or close to the range of maximum effect. But whether this difference was due to variation in the reaction, to a peculiarity in the rubber membrane, or to leakage of the injected material, cannot be definitely stated. In the Smith and Mair barium hydroxide saponification (Nos. 168-170), the first experiment gave no reaction, and the last two, reactions in the range of moderate effect. Here again the difference might be due to differences in the effect of the treatment upon the substance (though this seems improbable, in view of the close agreement of the results for unsaponifiable matter), to leakage of injected material, or to variation in the reaction. In none of the cases of duplicate tests of the same emulsion, or of the series for reaction as a function of dosage, was there any overlapping of the ranges of slight and moderate effect.

In cases where the difference in reaction of the original and treated materials is too small to permit definite conclusions to be drawn, two further procedures can be used to give more definiteness. Here, as in any case of random variation, certainty is increased by increasing the number of experiments. In this work, all important experiments were done in duplicate, and several were performed many times. The bromine treatment, for instance, was done five times. Agreeing results, under these conditions, give entire certainty. The other method, which, however, cannot always be used, is that of changing the conditions of the experiment so as to emphasize the effect produced by whatever treatment the active substance is undergoing. For example, the reaction after treatment with aqueous sulfuric acid for one hour (No. 159) was in the range of moderate effect. After treatment for one day (Nos. 157 and 158) or four days (No. 156), the effect was only slight.

In still other cases, conclusions can be drawn from the internal consistency of various sorts of experiments. Thus, in the case of benzin, the solubility, dialysis, and partition experiments all show that the active substance is only slightly soluble (in true solution) in benzin. Consequently, there is no reason to doubt the result of any one experiment. Again, in the tests for the carbonyl group, four different methods were used, all of which gave products which caused reactions in the range of moderate effect. Again there is no reason to doubt the result of any single test.

In general, conclusions can be drawn from the result of a single test only in certain cases. If several tests be made, definite results can be obtained in other cases. There appears to be no reason why, if sufficient tests be made, fairly definite conclusions cannot be obtained in every case, if dilutions be used which give reactions in the range of moderate effect.

If highly concentrated materials are tested in the ordinary dilution of 25 mg. per cc., and give reactions in the range of maximum effect, obviously no conclusions can be drawn from the tests as to the relative concentrations of active substance. But if the dilution be increased until the reactions fall within the range of moderate effect, where sufficient change of concentration does result in change in amount of effect, then the results of the tests can be interpreted as has been shown above. There is, of course, no limit to the extent to which this dilution can be carried, and so the method may be applied to any preparation of greater concentration than that necessary for reactions in the range of moderate effect.

The present method appears to have two advantages over that of Frank: it is more rapid, and its results are expressed in terms of an objective standard, so that the results obtained with it by different experimenters can be compared. It is possible, however, that Frank's method, on account of the integrating effect of using four injections, may give more uniform reactions than the present one. It is also possible that intraperitoneal injections may achieve this, on account of more uniform absorption conditions.

The injections, in general, were borne well by the animals. There have been a total of three deaths out of 142 animals injected, all with emulsions sterilized by the Arnold method. These, in the gross, appeared due to bacteremias. Only 23 of the animals lost weight. A single animal was injected intraperitoneally, without any ill effects being observed. In this case, the uterus growth was less than in a corresponding subcutaneous injection.

D. Solubility Experiments

In connection with the solubility experiments, it must be remembered that the solubility of fat-like substances is greatly influenced by the presence of other substances. Neutral fat, for example, is practically insoluble in alcohol, but soluble in alcoholic solutions of free fatty acids. Consequently, the results obtained in these experiments apply with certainty only to the particular mixtures ("Stock I" and "Stock II") with which the experiments were conducted. More general results, however, can be obtained by dialysis experiments.

Using "Stock I", the active substance was found to be soluble in benzene, chloroform, carbon tetrachloride, water, absolute alcohol, ether, ethyl acetate, carbon disulfide, and pyridine. With glacial acetic acid, the soluble portion was found to be slightly active physiologically. The grease insoluble in water was much more active than the water-soluble portion. The substance was insoluble in benzin. Using "Stock II," the active substance was soluble in benzene, acetone, and 95% and 50% alcohol, and somewhat soluble in glacial acetic acid and benzin. "Stock II" dissolved completely in carbon disulfide and pyridine, and in hot acetic anhydride. It dissolved completely in acetyl chloride to form an opalescent solution. The portion insoluble in 50% alcohol was active; those insoluble in 60% alcohol and glacial acetic acid were slightly active.

E. Partition Experiments

Partition experiments were carried out with various pairs of solvents to develop methods of concentrating the active substance. With benzene and water, the benzene phase was active, and the aqueous inactive. With benzin and water, the most of the active material appeared to be in the benzin phase; when the aqueous phase contained a good deal of colloidal material, as in extracting with benzin the emulsion obtained by evaporating the alcohol from the first alcoholic extract of placentas, the aqueous phase contained much more active material; in one case the benzin layer showed none at all on test. This made it appear that solutions of the active substance in both water and benzin were mainly colloidal, which was subsequently confirmed by dialysis experiments. With benzin and 75% alcohol, the first benzin extract showed no activity, but the second benzin extract of the alcoholic layer was somewhat active. This difference was probably due to the proportionately larger amount of inert material in the first extract. With benzin and 50% alcohol, no activity was found in the benzin,

phase; the alcoholic phase was active. With benzene and 75% alcohol, no satisfactory separation was obtained.

F. Dialysis Experiments

Using rubber membranes, according to the method of Gies[34] and Rosenbloom[35], the substance was found to dialyse in acetone, benzene, ether, and chloroform solution. In benzin and carbon tetrachloride solution, only slight activity was found in the dialysate. With collodion membranes, the substance dialysed in 75% and 62½% alcohol solution. Practically nothing came through in 50% alcohol solution.

From these results, it appears probable that the active substance dissolves in water and 50% alcohol only as a colloidal solution; that in benzin and carbon tetrachloride it is present to a slight extent in true solution, the remainder being colloidal; that in benzene, chloroform, acetone, ether, and strong alcohol it is present in true solution, while in intermediate strengths of alcohol it is present partly in colloidal, and partly in true solution.

G. Effect of Conditions and Reagents

The effect of numerous conditions and reagents upon active preparations was tried, in order to find out what treatments could be used without destroying the substance, to get some notion of its chemical behavior, and, if possible, to prepare some easily purified derivative, from which the active substance might be recovered, this last, however, without success.

Emulsions of active materials in mucilage of tragacanth were found to stand sterilization, by either the Arnold method or autoclaving, without apparent change. The activity was not destroyed by heating "Stock II" for 14 days in a closed tube in the steam bath, or by heating its alcoholic solution or aqueous emulsion similarly for 4 days. The activity was destroyed by heating on the steam bath for 7 days, exposed to the air.

A mucilage of tragacanth emulsion was found to have lost its activity largely after standing in a cotton-stoppered bottle for five weeks. The activity of an alcoholic solution was found to have decreased markedly after six weeks' standing. Five weeks' standing did not appear to affect a benzene solution, however. Two and a half years' standing in the solid condition in a corked flask failed to destroy the activity of one preparation.

In the preliminary work, active materials were obtained after treatment with both acid and alkali. From this it was concluded that these reagents did not affect the active substance. But in the later work, where

the effect of a certain amount of active material was compared with the effect of an equal amount after treatment with the reagent, the activity was found to be greatly reduced by acid treatment, and in some cases could not be detected at all after alkali treatment. The experiments (Nos. 156-159) show clearly the progressive diminution of the physiological effect as the time of acid treatment was increased. No active material was recovered after acidifying the alkaline solution or making alkaline the acid solution; consequently the destructive effect is not due to either basic or acidic properties of the active substance. By the Smith and Mair barium hydroxide saponification, conflicting results were obtained. Since by this method cerebrosides are practically unaffected, one may conclude that the active substance is hydrolysed less easily than neutral fat and lecithin, but more easily than the cerebrosides.

From these results it appeared that the substance was of an ester-like nature. An attempt was made to hydrolyse it with soy-bean lipase, but without definite effect.

The activity was also destroyed by treatment with nitrous acid, bromine, and cold alkaline permanganate. Since boiling brominated "Stock III" with zinc and glacial acetic acid did not restore the activity, it is probable that substitution as well as addition takes place during the bromine treatment. It was not destroyed by acetyl chloride, boiling acetic anhydride, zinc and glacial acetic acid, sodium carbonate in aqueous solution, sodium bisulfite, cadmium chloride, fuller's earth, phenylhydrazine, hydrocyanic acid, hydroxylamine, or semicarbazide. "Stock III" contained no nitrogen, sulfur, or halogens. These results indicate that the substance contains neither the carbonyl, amino, imino, nor hydroxyl grouping. It is probably an unsaturated compound of ester-like nature.

H. Progress toward the Preparation of the Pure Active Substance

As Herrmann gives no criteria of purity for his substance, his claim to have secured the active substance in pure form must be considered as resting on rather dubious grounds. Since cholesteryl palmitate was found in the active fraction from the first distillation, and was separated only by its failure to dissolve in alcohol, in which it is somewhat soluble, it was probably present also in the final product. Nor is there any reason to think that the cholesterol had been entirely removed by Herrmann's procedure. In the present work, it has been found to be a most persistent impurity. The strong cholesterol test given by the "pure substance" can be explained by the presence of either of these substances.

In the present work, by crystallizing "Stock III" in the ice-box from

90% acetone, a pale yellow substance was obtained which, when heated on mercury, softened at 52° and melted at 56°. On recrystallization from 83% acetone, a white, crystalline substance was obtained which softened on mercury at 56° and melted at 57° (corrected). It gave only a pale emerald-green color by the Liebermann-Burchard test for cholesterol. It was moderately active physiologically. A repetition of this work with larger quantities, however, gave a substance which melted at 54.2°-55.2° (corrected), and which on test proved to be entirely inactive. The mother-liquor from the last crystallization of this substance was slightly active. It must, therefore, be concluded that the activity of the earlier preparation was due to insufficient purification.

For the further concentration of "Stock III", it seems probable, on the basis of the present work, that the most serviceable method will be that of partition between benzin and 75% alcohol, using the method after the manner of fractional crystallization.

III. EXPERIMENTAL

A. Preliminary Work

The physiological tests throughout the preliminary work were made by Dr. Robert T. Frank.

Preparation of "Stock A"—Lee's Method.—No. 1. The cords and membranes were cut off from the placentas and all blood clots removed. After chopping up the organs in a meat grinder the pulp was stirred with alcohol and let stand for several days, after which it was pressed out in a meat press. The juice was then evaporated to dryness on the steam bath and the residue, together with the pressed out placental pulp, was extracted for at least a week with alcohol in a Soxhlet extractor of about 500 lbs. capacity. The extract was then evaporated to dryness on the steam bath; the residue has been designated as "Stock A."

Fractionation of "Stock A"—Method of Herrmann.—No. 2. "Stock A" was crushed and extracted in a mortar with ether until nothing more dissolved. The extract was filtered and evaporated on the water bath. It tested +1½. It was then treated with about three and one-half times its volume of acetone. The precipitate was filtered off and dissolved in ether, and the solution was evaporated to dryness. Acetone was added to this, and the mixture was again filtered. The precipitate was again dissolved in ether, evaporated, and extracted with acetone. The acetone filtrates were combined, evaporated to a small volume, and cooled in the ice-

chest. The solution was filtered with suction and evaporated on the steam-bath. After adding an equal volume of alcohol it was cooled in the ice-box. It was then filtered and the residue washed with alcohol. The solution was evaporated on the steam bath, and an equal volume of methyl alcohol added. The mixture was cooled in the ice-box, filtered, and the precipitate washed with methyl alcohol. The filtrate was evaporated. The residue was distilled in vacuo at a pressure ranging from 10 to 20 mm. Fractions were obtained boiling at 160° to 357°, all of which were somewhat active.

Unsuccessful Attempts at Concentration of Active Material in "Stock A."—No. 3. "Stock A" was emulsified with methyl alcohol and ether was added. After filtering off the voluminous precipitate, the filtrate was evaporated on the steam bath. On shaking with water and ether an emulsion formed. A separation was obtained by the addition of alcohol and salt. The ether-alcohol extract was evaporated on the steam bath. It tested +1.

No. 4. "Stock A" was shaken with water and ether. On settling, four layers formed: at the top the ether extract, then emulsion, then an aqueous layer, and a layer of solid at the bottom.

No. 5. "Stock A" was emulsified in alcohol and ether was added. The liquid was filtered and the filtrate evaporated on the water bath. The residue tested +1½.

No. 6. "Stock A" was emulsified with water. It was, however, found impossible to filter the material.

No. 7. "Stock A" was boiled with water. While hot it could be filtered with suction, giving a filtrate containing small globules of grease which could not be removed by further filtration.

No. 10. "Stock A" was emulsified with glacial acetic acid and filtered. A sample of the light brown filtrate on dilution with five volumes of ether gave a brown precipitate, insoluble in alcohol, and soluble in water. The remainder of the filtrate was evaporated on the water bath. On test it was inactive.

Preparation of "Stock B" and 95% Alcohol Dialysis.—No. 11. "Stock A" was boiled with water, and alcohol and ether were added. After filtration, the solution was evaporated to dryness. The residue tested +2½. The term "Stock B" will be used hereinafter to designate the material prepared in this fashion. This was dissolved in 95% alcohol and dialysed through a collodion bag into 95% alcohol. The dialysate tested +1½. Subsequent dialysates tested +2.

Alcoholic Sulphuric Acid Treatment.—No. 13. About 5 g. of "Stock B" were shaken with a solution of 10 cc. of sulfuric acid in 40 cc.

of 95% alcohol. Water was added and the solution was extracted with ether twice. The extract was washed with water twice and evaporated on the steam bath. The residue tested +2½. Two months later the same material, which had been standing in a beaker in the laboratory, was inactive on test.

Alcoholic Potash Treatment.—No. 14. Fifteen g. of potassium hydroxide were dissolved in 60 cc. of 95% alcohol. To 50 cc. of this solution 5 g. of "Stock B" were added; the mixture was then boiled under a reflux condenser for half an hour. Twice the volume of water was added and the mixture was extracted with ether twice. The extracts were washed twice with water and evaporated on the steam bath. After dissolving the residue in ether, the solution was filtered and evaporated on the steam bath. The residue was extracted with cold alcohol; after filtration, the solution was evaporated as before. The residue tested +2.

No. 15. About 25 g. of "Stock B" were evaporated on the steam bath over night with alcoholic potash. The residue was taken up in water, some alcohol was added, and the mixture was extracted with ether five times. The extracts were washed with water four times and evaporated to dryness. The residue was inactive on test. On boiling with acetic anhydride under a reflux condenser for two hours no hydrocarbons separated.

No. 16. "Stock B" was evaporated on the steam bath over night with alcoholic potash. After the addition of alcohol and water the solution was extracted twice with ether. The ether extracts were washed once with water, and the washing was extracted twice with ether. These extracts, together with the former ones, were washed with water three times and evaporated on the steam bath. The residue tested +2.

Aqueous Solubility.—No. 17. "Stock B" was boiled with water. An orange solution was obtained which was filtered and evaporated on the steam bath. The residue was inactive on test.

Aqueous Dialysis.—No. 18. "Stock B" was boiled in water and the emulsion filtered. The red-brown solution after cooling was placed in a collodion bag with water outside. The dialysate was removed 8 days later and evaporated on the steam bath. The residue was extracted with alcohol and the filtered extract evaporated. On test, the residue was inactive.

Solubility in 50% Alcohol.—No. 19. "Stock B" was emulsified with 50% alcohol and centrifuged. It separated into six layers, of which the principal ones were the original grease, the alcoholic solution, and a yellow precipitate. The grease layer was shaken with 50% alcohol and centrifuged, and this was repeated until it had all been converted into precipitate and alcoholic extract. The residue, after evaporation of the 50% alcoholic

solution, tested +2. The yellow precipitate was inactive. The alcoholic extract on evaporation had formed a hard glassy mass. Three months later this tested +1¾ or +2.

Solubility in 50% Acetone.—No. 20. A similar experiment was made with "Stock B" and 50% technical acetone. The 50% technical acetone solution on evaporation tested +1½.

Solubility in Ethyl Acetate.—No. 21. "Stock B" was extracted with ethyl acetate twice. The filtered solution was evaporated on the steam bath. The residue tested +2. The material insoluble in ethyl acetate tested +½. Three months later, the ethyl-acetate-soluble fraction tested +½ to ¾.

Solubility in 78° Benzin.—No. 22. "Stock B" was shaken with 78° benzin and the emulsion centrifuged. The solution was evaporated on the steam bath, taken up in alcohol, and the alcoholic solution evaporated. It tested + or —. The residue obtained on centrifuging was dissolved in alcohol and the alcoholic solution evaporated on the steam bath. It tested +2.

Solubility in 25% Alcohol.—No. 23. "Stock B" was shaken with 25% alcohol and centrifuged. The solution was filtered and evaporated on the steam bath. It tested +1.

No. 24. "Stock B" was stirred with 50 cc. of alcohol and 50 cc. of water were added. The solution after filtration was evaporated on the steam bath. Fifty cc. of alcohol were added and the mixture was warmed and stirred until solution was complete, when 150 cc. of water were added and the solution was filtered. The filtrate was evaporated on the steam bath; the residue tested +2. The precipitate was dissolved in alcohol, and the filtered solution was evaporated on the steam bath. The residue tested +3.

No. 25. "Stock B" was dissolved in 200 cc. of 95% alcohol, and 180 cc. of water were added. Centrifuging did not effect a separation, so the emulsion was left in a separatory funnel over night. The next day the lower alcoholic layer was drawn off and filtered. Its volume was 310 cc. An equal volume of water was added and the solution was filtered. The insoluble material was dissolved in alcohol, and the filtered solution evaporated on the steam bath.. The residue tested +3.

Reaction of "Stock B."—No. 26. Fresh ground placentas were stirred with alcohol and permitted to stand. The mixture was pressed out in a meat press and the pulp then extracted according to Lee's method. "Stock B" prepared from this material tested +½.

Alcoholic Potash Saponification, followed by 50% Alcohol

Extraction.—No. 27. To some of this "Stock B" alcoholic potash was added. The solution was evaporated on the steam bath for several hours. After dissolving the residue in alcohol and water, the solution was extracted with ether. The extract was washed with water and evaporated on the steam bath. The residue tested ± or —. After adding alcohol, the mixture was warmed on the steam bath, cooled, and filtered with suction. The cholesterol was washed several times with alcohol, and the filtrate was evaporated on the steam bath. The residue tested +1. After removing a second crop of cholesterol as before, the material tested +2. It was shaken with 50% alcohol and centrifuged. The alcoholic solution was filtered and evaporated on the steam bath. A solution containing 70 mg. of the residue in 10 cc. tested +2.

Acidification of Alkaline Solution.—No. 28. Another sample of "Stock B" from No. 26 was shaken with alcoholic potash. After the addition of water the solution was extracted with ether. The aqueous residue after the ether extraction was acidified with dilute sulfuric acid and extracted with ether twice; the extract was washed with water, filtered, and evaporated on the steam bath. On test, the residue was inactive.

Ether Extract of Dried Placentas.—No. 29. Placental pulp which had been dried at 37° under reduced pressure was extracted with ether in a Soxhlet extractor, and the extract evaporated to dryness. It tested +½. Two repetitions of this both gave tests of +½.

Aqueous Extraction of Fresh Placentas.—No. 30. To 1580 g. of fresh ground placentas were added 4 liters of water containing 1% of acetic acid. After shaking, the mixture was heated on the steam bath over night, the temperature ranging from 50° to 65° C. The mixture was then strained through cheesecloth, and the liquid obtained was heated on the steam bath to 87° for about an hour. It could not be filtered. It was evaporated on the steam bath under reduced pressure to a volume of 720 cc. To it were added 1800 cc. of 95% alcohol, and the mixture was well shaken. After standing over night it was filtered. One-half of the filtrate was evaporated under reduced pressure to 165 cc. and filtered. It was made alkaline with ammonia, an excess of 5 cc. being added. After cooling, it was filtered, but nothing was obtained. The other half of the alcoholic filtrate was evaporated on the steam bath, and the residue was extracted with hot 95% alcohol. The extract was filtered and evaporated to dryness. This material did not dissolve in absolute alcohol. It was extracted with alcohol and the extract filtered and evaporated on the steam bath. The material still did not dissolve completely in alcohol. It was treated with hot alcohol and twice the volume of water added, then was

extracted with ether repeatedly. After evaporating the extract on the steam bath, the residue tested +1½.

No. 31. The placental pulp which had been extracted with water in No. 30 was dried on the steam bath, crushed in a mortar, and extracted with ether in a Soxhlet extractor. The ether extract was filtered and evaporated on the steam bath. On test it was inactive. Two days later a solid material was found to have separated in the extract. It was taken up with cold 95% alcohol and filtered. The filtrate was evaporated on the steam bath. The residue tested +½.

No. 32. To 6215 g. of fresh ground placentas were added 8 liters of water and 80 cc. of glacial acetic acid. The mixture was boiled for one-half hour and strained. The pulp was pressed out in a meat press, and the liquor was evaporated on the steam bath to a moist lumpy state. After the addition of an equal volume of alcohol it was heated to boiling. The mixture after cooling was extracted with ether three times. After evaporating the extracts, the residue was extracted with cold alcohol, and the filtered extract was evaporated on the steam bath. Repeated extraction with alcohol, filtration, and evaporation failed to give a material which was completely soluble in alcohol. Finally 40 cc. of alcoholic potash were added and the mixture was heated on the steam bath over night. The residue was dissolved in water and extracted well with ether; the extracts were evaporated. The residue gave the Liebermann-Burchard test for cholesterol. It was extracted with 95% alcohol, and the extract was filtered and evaporated. The residue (0.14 g.) tested +3.

No. 33. A similar experiment again yielded a residue testing +3.

Alcoholic Extract of Fresh Placentas.—No. 34. Fresh ground placentas were stirred with alcohol. Several days later the juice was pressed out and boiled down over a flame to a thin mush. Alcohol and ether were added, and the extract was decanted and filtered. After repeating this three or four times, the extract was evaporated on the steam bath. The residue tested +2½ or 3. Alcoholic potash was added and the mixture was evaporated on the steam bath over night. The residue was taken up in water and alcohol and extracted four times with ether. The extract was washed with water three times and evaporated on the steam bath; the residue tested +2½. It was dissolved in warm alcohol and cooled. After filtering off the cholesterol the solution was evaporated on the steam bath. The residue tested +2½.

No. 35. Filtered press juice was evaporated on the steam bath and finally boiled down over a free flame. The residue was taken up in water and extracted with ether. The extract was evaporated on the steam bath; the residue tested +1¾.

No. 36. The alcoholic press juice from fresh ground placentas was boiled down and extracted with ether and alcohol. The extract was evaporated for 40 hours on the steam bath with alcoholic potash. The aqueous solution of the residue was extracted with ether, and the extract was evaporated on the steam bath. After shaking the residue with 50% alcohol, the centrifuged solution was filtered. The residue was again shaken with 50% alcohol, and the solution centrifuged and filtered as before. The filtrate was evaporated on the steam bath; the residue (90 mg. in 10 cc. of alcohol) tested +3.

Physiological Tests with Varying Concentrations.—No. 37. Filtered alcoholic press-juice was evaporated on the steam bath; the residue was taken up in water and alcohol and extracted with ether three times. After filtering, the ethereal extract was washed with water twice, and was then evaporated on the steam bath. The residue was tested in various dilutions. A 12.5% solution tested +2½ or 3. A 6.3% solution tested +3. A 2.5% solution tested +3. A 1.25% solution tested +2.

No. 38. Ten lbs. of fresh ground placentas were stirred well with four liters of 95% alcohol and let stand for two days. In this time some alcohol evaporated. The addition of two liters more gave what appeared to be the same consistency as the original. Another liter of alcohol was stirred in and the material was pressed out. The juice was evaporated on the steam bath. Water was then added and the mixture was warmed. After adding alcohol, the solution was extracted with ether three times. The combined extracts were washed with water twice. The washings were evaporated on the steam bath; the residue tested +2. The ethereal solution was evaporated on the steam bath; the residue weighed 12.14 g. Various dilutions of this material were tested. A 25% solution tested +3. A 12.5% solution tested +2½. A 5% solution tested +2. A 2.5% solution tested +2. A 1.25% solution tested +2 to 2½. A 0.5% solution tested +1.

No. 39. Alcoholic press juice was evaporated on the steam bath over night to a volume of about three-fourths of the original. It was then filtered and boiled down over a flame. After extracting with alcohol and ether, alcoholic potash was added to the extract and the whole evaporated on the steam bath. The next day water was added and the solution extracted with ether four times. After washing twice with water the extract was evaporated on the steam bath. The residue was taken up with 50% alcohol and centrifuged; this extraction was repeated twice. After finding it impossible to filter the liquid, it was evaporated on the steam bath. The residue was taken up in 10 cc. of alcohol, then 10 cc. of water were

added slowly with stirring. The emulsion was centrifuged and the solution decanted. The residue was shaken with 11 cc. of alcohol and the solution diluted to 22 cc. with water and centrifuged. The supernatant liquid was combined with the previous solution and both were evaporated on the steam bath. The residue was tested in various dilutions. A 2.5% solution tested +2. A 0.5% solution tested +2. A 0.1% solution tested +1¾. A 0.02% solution on test was inactive.

Repeated Alcoholic Extractions of Fresh Placentas.—No. 40. Ten lbs. of fresh ground placentas were stirred with 5 liters of 95% alcohol, let stand over night, and pressed out. The press cake was subjected to further extractions. Each extract was evaporated under reduced pressure and the residue taken up in water and extracted with ether.

First extraction: 5 l. alcohol, 1 day; 3.46 g. yield.
Second extraction: 5 l. alcohol, 2 days; 63.88 g. yield.
Third extraction: 4 l. alcohol, 2 days; 15-20 g. yield.
Fourth extraction: 4 l. alcohol, 3 days; 4.76 g. yield.
Fifth extraction: 4 l. alcohol, 3 days; 3.14 g. yield.
Sixth extraction: 5 l. alcohol, 4 days; 2.31 g. yield.

A similar experiment gave the following results:

First extraction: 5 l. alcohol, 29 days; 2.50 g. yield.
Second extraction: 5 l. alcohol, 2 days; 48.33 g. yield.
Third extraction: 5 l. alcohol, 4 days; 6.22 g. yield.

Fractionation of Second Alcoholic Extract of Fresh Placentas.—No. 41. Nineteen lbs. 3 oz. of fresh ground placentas were stirred with 9¾ liters of 95% alcohol. Two days later this was pressed out. The juice was discarded. The press-cake was broken up and stirred with 10 liters of alcohol. Two days later this was pressed out. The filtered juice was evaporated under reduced pressure, and the residue was taken up with water. The ether extract of this solution was evaporated on the steam bath and dried on the steam bath under reduced pressure. Yield, 80.5 g. This residue was taken up in hot alcohol. On cooling, some crystals formed, and an oil separated on the bottom of the beaker. After decanting the solution and crystals, the residue was again heated with alcohol and permitted to cool. This extraction was repeated several times, the solution and crystals being decanted each time from the residual oil. The oil tested +1. The crystals were filtered off and recrystallized. They resembled cholesterol and gave the Liebermann-Burchard test. The mother liquor from the crystals was evaporated under reduced pressure. Much material crystallized. These crystals melted below room temperature, so the liquid was cooled in the ice-box and filtered there on a folded filter. Suction

filtration did not work. The crystals were taken up in alcohol at room temperature. Some oil did not dissolve. This was extracted about four times with alcohol. It tested +1. The alcoholic solutions were permitted to evaporate in the ice-box. Four days later the crystals were filtered off. They were then shaken with ether and the solution was evaporated on the steam bath under reduced pressure. Yield, 8 g. On test, the residue was inactive.

No. 42. Nine and one-half lbs. of fresh ground placentas were stirred with 5 liters of alcohol. After two days the juice was pressed out and discarded. The cake was broken up and stirred with 5 liters of alcohol. After four days the juice was pressed out and evaporated under reduced pressure. The residue was taken up in water and extracted with ether; the extract was evaporated on the steam bath. The residue was dried in vacuo on the steam bath; yield, 44½ g. This material was shaken repeatedly with 95% alcohol and the solutions were filtered. The filtrate separated into two phases. The upper liquid phase was decanted and evaporated on the steam bath under reduced pressure. Yield, 33 g. The residue tested +1½.

No. 43. Fifteen lbs. of fresh ground placentas were stirred with 8 liters of alcohol. After two days the juice was pressed out and discarded. The cake was broken up and stirred with 8 liters of alcohol. After two days the juice was pressed out, filtered, and evaporated under reduced pressure. The residue was taken up in water and the solution extracted with ether. The extract, after settling in the ice-box, was evaporated on the steam bath. The residue, after being dried on the bath in vacuo, was taken up in hot alcohol; after standing over night the solution was filtered and the filtrate was evaporated under reduced pressure on the steam bath. Yield, 37 g. The material tested +2.

Evaporation of First Alcoholic Extract of Fresh Placentas at Low Temperature.—No. 44. Fourteen lbs. of fresh ground placentas were stirred with 7 liters of alcohol. After two days the juice was pressed out, filtered, and evaporated under reduced pressure. One-half of the residue was taken up in water and extracted with ether. The extract was evaporated on the steam bath and the residue was dried in vacuo. It was then taken up in a little ether, evaporated on the steam bath, and dried on the bath for only a few minutes. Yield, 0.73 g. Two dilutions of the residue were tested: a 2.5% solution tested +3; a 0.1% solution tested +1.

B. Preparation of Stock Materials.

"Stock I": Placentas were obtained through the courtesy of the Sloane Hospital for Women and the Jewish Maternity Hospital. After delivery, they were kept in an ice-box, from which they were removed three times a week. The membranes and cords were cut off and blood clots removed, after which the organs were ground in a meat chopper. Ten lbs. of pulp were stirred with 7 liters of 90% alcohol. After standing over night the pulp was squeezed as dry as possible in an Excelsior meat-press, using hydraulic press-cloth. Above the bottom plate of the press was used an iron plate punched with 1/8" holes. The yield of juice ran from 7.1 to 8.8 liters; the alcoholometer reading was from 56 to 62%. After filtering through paper, the extract was evaporated under reduced pressure in a battery of six three-liter round-bottom flasks which were set in a water-bath kept at 40°. The condenser was cooled with ice-water. To prevent foaming, about 200 cc. were placed in each flask at the start; the rest was added gradually during the evaporation. When the volume of the extract was less than three liters, it was transferred to a single flask and evaporated further with an ice-water condenser and May-Nelson pump. For "Stock I", the evaporation was carried clear to dryness. The residue was shaken with 30 cc. of water until all, or nearly all, was emulsified. After adding 300 cc. of 95% alcohol and shaking well, the solution and residue were decanted as completely as possible. The residue in the flask was emulsified with 10 cc. of water; the emulsion was shaken with 100 cc. of 95% alcohol, and the liquid added to the first liquor. After settling over night, the clear, supernatant liquid was decanted through a filter. On evaporation at 40° under reduced pressure, "Stock I" was obtained. The yield from ten pounds of fresh placentas ran from 40 to 43 grams. The material was of a greasy, gummy nature, orange to red in color. The last evaporation was rendered difficult by a marked tendency toward foaming.

"Stock II": This, the best method developed, was the same as that for "Stock I" as far as the conclusion of the first evaporation. For "Stock II" this evaporation was stopped when the liquid had a volume of less than one liter. It was then shaken with 100 cc. of benzene, and the mixture permitted to stand over night. The bottom layer of sludge and the next layer of aqueous solution were drawn off together, and the two upper layers of emulsion and benzene solution were centrifuged. The aqueous solution was re-extracted with 100 cc. of benzene, the separation taking place much quicker this time. A comparatively small amount of material was extracted by this second treatment, as was shown by the much lighter color of the extract. This second extract was then shaken well with the

emulsion separated by centrifuging the first extract, and the whole was centrifuged. The evaporation of the combined benzene solutions under reduced pressure gave "Stock II". The yield from ten pounds of fresh placentas ran from 5 to 6½ g. The material resembled "Stock I" in appearance. For methods of using in physiological tests, see "C. Physiological Tests".

In most of the experiments, only a portion of the benzene solution was evaporated at a time. The flask containing the solution was heated in a water-bath at 40°; another flask in an ice-water bath served as a condenser. After the evaporation was concluded, the condenser was replaced by a similar flask containing strong sulfuric acid. The heating in vacuo was continued until after an hour's heating the weight was within 0.03 g. of the previous weight. Wherever in this work preparations are said to have been "dried", this is the procedure referred to.

"Stock III": "Stock II" (from 4 to 10 g.) was shaken with acetone. After decanting the solution, the residue was dissolved in ether, precipitated with acetone, and the solution decanted. This was repeated several times. The combined acetone solutions were evaporated to dryness, taken up in the minimum quantity of acetone, and poured into a heavy rubber condom suspended in a 250 cc. Erlenmeyer flask containing 200 cc. acetone. After standing at least five hours, the dialysate was decanted and replaced by a fresh 200 cc. portion of acetone. This was continued until no more material came through. The dialysates were heated on the steam bath till almost all the acetone had boiled off, and were then evaporated under reduced pressure at 40°. The dialysate contained, in addition to the active material, cholesterol. This was largely removed by the 62½% alcohol dialysis which followed. The acetone dialysate was dissolved in the minimum quantity of warm 95% alcohol. To this solution was added sufficient water to dilute the alcohol to 62½%. The emulsion was poured into a collodion sac suspended in a 250 cc. Erlenmeyer flask containing 200 cc. of 62½% alcohol. The flask was then placed in a water-bath at 40°. After standing over night, the dialysate was decanted and replaced by a fresh 200 cc. portion of 62½% alcohol. The dialysation was continued until the residue of one dialysate on evaporation was not over 0.07 g., the amount of cholesterol which dialysed under similar conditions. The dialysates were evaporated under reduced pressure at 40°. The residue constituted "Stock III". The yield of this varied from 12 to 27% of the "Stock II" used.

Second Extraction of Placentas.—No. 45. The press cake from the first extraction of 5 lbs. of fresh ground placentas was broken up and stirred with 3.5 liters of 57% alcohol. The next day the alcoholic solution

was pressed out and "Stock I" prepared as usual. The total yield was 4.6 g. A rabbit weighing 327 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 403 g. Uterus not congested and only slightly, if at all, enlarged. Empty body, 259 g. Dried uterus, 0.0123 g. Normal, 0.0070-0.0120 g.

Autolysis before Extraction.—No. 46. Fresh placentas were autolysed for five days with 1¼% toluene. "Stock I" was then prepared from the material as usual. The precipitate with alcohol in preparing the final solution for "Stock I" was twice as large as usual.

C. Physiological Tests.

In the preliminary work the physiological tests were made by Dr. Robert T. Frank. The material to be tested was dissolved in absolute or 95% alcohol to make a 2.5% solution. One cc. of this was injected every two days and the rabbit killed two days after the fourth injection. While the absorption of the injected material was quite rapid, this method had the objection that the alcohol caused necrosis of the tissues and resulting abscesses. In the later work, instead of the alcoholic solution an emulsion of the material to be tested in U. S. P. mucilage of tragacanth containing 0.4% of tricresol was used. Most of the materials emulsified fairly rapidly with the mucilage. Where emulsification did not take place in the cold, shaking and warming frequently effected it. Shaking the mixture with glass beads also aided. In some cases the substance appeared to be a high-melting crystalline compound and would not emulsify under these conditions. In such cases a few drops of almond oil were added to the material and the mixture was dissolved in ether, which was then evaporated. The resulting material emulsified easily.

Sterility of the emulsions was secured by three different procedures. In the early part of the work, before it was known what effect high temperatures had upon the active substance, the emulsions were prepared aseptically. The material to be tested was permitted to stand over night with 50% alcohol and this was then evaporated aseptically under reduced pressure at 40°. The residue was then emulsified with sterile mucilage of tragacanth, using sterile glass beads where necessary. Later on it was found that heating the emulsion for 20 minutes on three successive days at 100° in an Arnold sterilizer did not destroy the activity. This method, being much easier than the former one, was thereupon adopted. Still later it was found that the emulsion could be autoclaved for one-half hour at 15 to 20 lbs. pressure without apparently reducing the activity. This,

being the more certain method of sterilization, was consequently adopted for the remainder of the work. In both of the last two methods the emulsion to be sterilized was sealed in an ampoule. The site of injection was shaved and sterilized with tincture of iodine. Tuberculin syringes graduated to 0.01 cc. were used. The injections were at first made by inserting the needle subcutaneously at a point well down the flank of the animal and running it up toward the head. Toward the end of the work certain discordant results led to the suspicion that possibly some injected material had escaped from the punctures. In several cases no unabsorbed residue of injected material was found on killing the animal. Thereafter the injections were made by inserting the needle at the median line of the animal and pushing it into the axilla. In order to prevent any possible loss of active material the puncture was sealed with collodion. In several cases where the animal had been injected by this procedure no residue of unabsorbed injected material was found at autopsy. Consequently there is no definite reason to suspect the earlier procedure of having been less accurate than the later one. It appears more probable that the divergent results were simply due to individual variations on the part of the animals and the position of the injected material.

After killing the animals, usually with ether, the uterus was carefully dissected away from the broad ligament. The tubes were cut off at the point of union with the horns, and the vagina was cut through at its point of attachment to the bladder. The uterus (and vagina) was then dried to constant weight on a Petri dish in a vacuum dessicator containing sulfuric acid. The digestive tract was removed from the animal, starting at a point just above the stomach, and ending at the point of attachment to the vagina. The remaining portion of the animal, designated as the "empty body", was weighed.

Reaction as a Function of Time.—To fix the period after injection at which the animal should be killed, three animals were injected with the same amount of active material and killed after 2, 4, and 8 days, respectively.

Two Days.—No. 47. A rabbit weighing 585 g. was injected with 1 cc. of 2.38% emulsion of "Stock I" (aseptically prepared). After 2 days, 598 g. Uterus congested and enlarged. Empty body, 443 g. Dried uterus, 0.0147 g. Normal, 0.0085-0.0120 g.

Four Days.—No. 48. A rabbit was injected with 1 cc. of 2.38% emulsion of "Stock I" (aseptically prepared). After 4 days it was killed. Uterus congested and enlarged. Empty body, 435 g. Dried uterus, 0.0391 g. Normal, 0.0080-0.0120 g.

Eight Days.—No. 49. A rabbit was injected with 1 cc. of 2.38% emulsion of "Stock I" (aseptically prepared). After 8 days, 657 g. The uterus showed local congestion. Empty body, 479 g. Dried uterus, 0.0219 g. Normal, 0.0115-0.0165 g.

Duplicate Tests.—On several occasions different animals were injected with the same amount of the same emulsion. For instances of this see No. 59, No. 115, No. 150, and No. 191.

Reaction as a Function of Dosage. First Series.—A series of rabbits were injected with emulsions of "Stock I" of varying concentrations in order to determine what function the reaction was of the amount of active material.

No. 50. A rabbit weighing 414 g. was injected with 1 cc. of 4.76% emulsion (aseptically prepared). After 4 days, 436 g. Uterus much congested. Empty body, 315 g. Dried uterus, 0.0528 g. Normal, 0.0070-0.0120 g.

No. 51. A rabbit weighing 425 g. was injected with 1 cc. of 2.38% emulsion (aseptically prepared). After 4 days, 474 g. Empty body, 336 g. Dried uterus, 0.0308 g. Normal, 0.0070-0.0120 g.

No. 52. A rabbit was injected with 1 cc. of 0.95% emulsion (aseptically prepared). After 4 days, 631 g. Empty body, 413 g. Dried uterus, 0.0100 g. Normal, 0.0080-0.0120 g.

No. 53. A rabbit weighing 546 g. was injected with 1 cc. of 0.48% emulsion (aseptically prepared). After 4 days, 588 g. Empty body, 415 g. Dried uterus, 0.0095 g. Normal, 0.0080-0.0120 g.

No. 54. A rabbit weighing 506 g. was injected with 1 cc. of 0.24% emulsion. After 4 days, 577 g. Empty body, 397 g. Dried uterus, 0.0108 g. Normal, 0.0080-0.0120 g.

Another set of emulsions was prepared about a week later from "Stock I" prepared from the same alcoholic solution as the previous one. Consequently the results are nearly comparable with the above.

No. 55. A rabbit weighing 584 g. was injected with 1 cc. of 19.0% emulsion. After 4 days, 607 g. Uterus much congested. Empty body, 388 g. Dried uterus, 0.0461 g. Normal, 0.0080-0.0120 g.

No. 56. A rabbit weighing 620 g. was injected with 1 cc. of 9.52% emulsion. After 4 days, 602 g. Uterus much congested. Empty body, 351 g. Dried uterus, 0.0547 g. Normal, 0.0070-0.0120 g.

Second Series.—Another series of emulsions was prepared from "Stock I" which had been prepared as rapidly as possible to avoid oxidative changes.

No. 57. A rabbit weighing 640 g. was injected with 1 cc. of 10% emulsion (prepared aseptically). After 4 days, 745 g. Empty body, 498 g. Dried uterus, 0.0347 g. Normal, 0.0120-about 0.0200 g.

No. 58. A rabbit weighing 387 g. was injected with 1 cc. of 5% emulsion (aseptically prepared). After 4 days, 458 g. Empty body, 275 g. Dried uterus, 0.0374 g. Normal, 0.0070-0.0120 g.

No. 59. Three rabbits were each injected with 1 cc. of 2.5% emulsion (aseptically prepared). (1) Before injection, 627 g. After 4 days, 700 g. Empty body, 486 g. Dried uterus, 0.0315 g. Normal, 0.0120—about 0.0180 g. (2) Before injection, 452 g. After 4 days, 510 g. Empty body, 340 g. Dried uterus, 0.0199 g. Normal, 0.0070-0.0120 g. (3) Before injection, 497 g. After 4 days, 612 g. Empty body, 404 g. Dried uterus, 0.0200 g. Normal, 0.0080-0.0120 g.

No. 60. A rabbit weighing 396 g. was injected with 1 cc. of 1.25% emulsion (aseptically prepared). After 4 days, 429 g. Empty body, 282 g. Dried uterus, 0.0125 g. Normal, 0.0070-0.0120 g.

Third Series.—No. 61. 0.85 g. of "Stock III" were emulsified to a volume of 8.5 cc. A rabbit weighing 371 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 394 g. A residue of unabsorbed injected material was found. Uterus deeply congested and greatly enlarged; tubes likewise. The ovaries were unaffected as far as the eye could see. The external genitals on pressure exuded mucus, and were congested. The vagina was greatly enlarged. Empty body, 241 g. Dried uterus, 0.1079 g. Normal, 0.0070-0.0120 g.

No. 62. A rabbit weighing 413 g. was injected with 0.50 cc. of the above emulsion. The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 464 g. The external genitals exuded mucus on pressure, and showed congestion. A residue of unabsorbed injected material was found. Uterus deeply congested and greatly enlarged; tubes likewise. The vagina also showed great enlargement. Empty body, 294 g. Dried uterus, 0.1065 g. Normal, 0.0070-0.0120 g.

No. 63. A rabbit weighing 452 g. was injected with 0.25 cc. of the above emulsion. The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 459 g. A residue of unabsorbed injected material was found. On pressure the external genitals did not exude mucus. Uterus deeply congested and greatly enlarged. The tubes and vagina were not as much affected as in Nos. 61 and 62. Empty body, 272 g. Dried uterus, 0.1083 g. Normal, 0.0070-0.0120 g.

No. 64. A rabbit weighing 351 g. was injected with 0.10 cc. of the

above emulsion. The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 381 g. A residue of unabsorbed injected material was found. Uterus congested and enlarged. Empty body, 241 g. Dried uterus, 0.0433 g. Normal, 0.0070-0.0120 g.

No. 65. A rabbit weighing 517 g. was injected with 1 cc. of 1.44% emulsion of "Stock III" (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 554 g. A residue of unabsorbed injected material was found. Uterus congested and enlarged. Empty body, 384 g. Dried uterus, 0.0675 g. Normal, 0.0080-0.0120 g.

No. 66. A rabbit weighing 428 g. was injected with 0.5 cc. of 1.44% emulsion of "Stock III" (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 465 g. A residue of unabsorbed injected material was found. The tip of the left horn of the uterus was congested and enlarged. Empty body, 314 g. Dried uterus, 0.0124 g. Normal, 0.0070-0.0120 g.

No. 67. A rabbit weighing 416 g. was injected with 0.25 cc. of 1.44% emulsion of "Stock III" (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 404 g. No residue of unabsorbed injected material was found. Uterus normal. Empty body, 249 g. Dried uterus, 0.0093 g. Normal, 0.0070-0.0120 g.

No. 68. A rabbit weighing 405 g. was injected with 0.10 cc. of 1.44% emulsion of "Stock III" (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 484 g. A residue of unabsorbed injected material was found. Uterus locally congested and enlarged. Empty body, 349 g. Dried uterus, 0.0192 g. Normal, 0.0070-0.0120 g.

Intraperitoneal Injection.—No. 69. A rabbit weighing 565 g. was injected intraperitoneally with 1 cc. of 1.44% emulsion of "Stock III" (autoclaved). The puncture was sealed with collodion. After 4 days, 569 g. A slight amount of material was found in one place attached to the intestine which proved to be an unabsorbed residue of injected material. Uterus congested and enlarged. Empty body, 401 g. Dried uterus, 0.0565 g. Normal, 0.0080-0.0120 g.

D. Solubility Experiments

In the solubility experiments, a weighed amount of "Stock I" or "II" was shaken with the solvent in the ratio of one gram of material to ten cubic centimeters of solvent. After centrifuging, the solution was

evaporated and the residue dried, weighed, emulsified, and tested physiologically.

Benzene.—No. 70. 6.55 g. of "Stock I" (dried, but not to constant weight) were shaken with 66 cc. of benzene and glass beads till all was emulsified. The solution was decanted, centrifuged, and evaporated under reduced pressure at 40°. The residue was dried: 0.45 g., 6.9%. A rabbit weighing 533 g. was injected with 1 cc. of 5% emulsion (prepared aseptically). After 4 days, 569 g. Uterus greatly congested and enlarged. Empty body, 403 g. Dried uterus, 0.0752 g. Normal, 0.0070-0.0120 g.

Benzin.—No. 71. 6.41 g. of "Stock I" were shaken with 64 cc. of Merck's U. S. P. benzin and glass beads till all was emulsified. The solution was decanted, centrifuged, and evaporated. Dried residue, 0.59 g., 9.2%. A rabbit weighing 420 g. was injected with 1 cc. of 1% emulsion (Arnold sterilization). After 4 days, 422 g. Uterus normal. Empty body, 262 g. Dried uterus, 0.0096 g. Normal, 0.0070-0.0120 g.

Chloroform.—No. 72. 6.37 g. of "Stock I" were shaken with 64 cc. of chloroform and glass beads till all was emulsified. The solution was decanted, centrifuged, and evaporated. Dried residue, 0.46 g., 7.2%. A rabbit weighing 395 g. was injected with 1 cc. of 1% emulsion (Arnold sterilization). After 4 days, 428 g. Uterus congested and enlarged. Empty body, 290 g. Dried uterus, 0.0285 g. Normal, 0.0070-0.0120 g.

Carbon Tetrachloride.—No. 73. 6.58 g. of "Stock I" were shaken with 66 cc. of carbon tetrachloride till all was emulsified. The solution was decanted, centrifuged, and evaporated. Dried residue, 0.50 g., 7.5%. A rabbit weighing 400 g. was injected with 1 cc. of 0.84% emulsion (Arnold sterilization). After 4 days, 408 g. Uterus enlarged, slightly congested. Empty body, 251 g. Dried uterus, 0.0345 g. Normal, 0.0070-0.0120 g.

Water.—No. 74. 6.69 g. of "Stock I" were shaken with 67 cc. of water. The solution was centrifuged and evaporated. Dried residue, 5.96 g., 89.1%. A rabbit weighing 633 g. was injected with 1 cc. of 5% emulsion (prepared aseptically). After 4 days, 704 g. Uterus congested and enlarged. Empty body, 487 g. Dried uterus, 0.0281 g. Normal, 0.0115-0.0205 g.

No. 75. In another experiment, 5.52 g. of "Stock I" were shaken with 55 cc. of water, and the emulsion was centrifuged. The supernatant grease was skimmed off and dissolved in ether, and the solution evaporated and dried. Residue, 0.77 g., 14%. A rabbit weighing 474 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days,

515 g. Uterus greatly congested and enlarged. Empty body, 337 g. Dried uterus, 0.0688 g. Normal, 0.0070-0.0120 g.

No. 76. An experiment was made to find out whether this method could be applied to the preparation of stock material. The evaporated alcoholic extract of fresh placentas was emulsified with water and the emulsion centrifuged. This procedure did not give promising results.

Glacial Acetic Acid.—No. 77. 6.35 g. of "Stock I" were shaken with 64 cc. of glacial acetic acid. The material emulsified readily, leaving a slight amount of white residue in the flask. A few glass beads were added and this residue was shaken loose. The solution was decanted and centrifuged. The clear dark yellow solution was evaporated under reduced pressure at 40°. Dried residue, 4.76 g., 75.0%. A rabbit weighing 475 g. was injected with 1 cc. of 5% emulsion (prepared aseptically). After 4 days, 511 g. The uterus was congested and portions of the horns were enlarged. Empty body, 322 g. Dried uterus, 0.0199 g. Normal, 0.0070-0.0120 g.

No. 78. In another experiment 4.93 g. of "Stock I" were shaken with 49 cc. of glacial acetic acid. The solution was centrifuged. The clear red solution was decanted from the cream-colored residue and evaporated under reduced pressure at 40°. A small amount of material was lost by foaming during the evaporation. Dried residue, 4.01 g., 81.4%. A rabbit weighing 660 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 656 g. The uterus was enlarged and slightly congested. Empty body, 492 g. Dried uterus, 0.0198 g. Normal, 0.0110-0.0200 g.

95% Alcohol.—No. 79. 4.47 g. of "Stock I" were shaken with 45 cc. of 95% alcohol and glass beads. The orange solution was decanted and centrifuged. The clear yellow solution with greenish fluorescence was then evaporated under reduced pressure at 40°. Dried residue, 2.16 g., 48.3%. This was not tested, as it was feared that the original "Stock I" had lost some of its activity through long standing.

Absolute Alcohol.—No. 80. 5.16 g. of "Stock I" were shaken with 52 cc. of absolute alcohol and glass beads. The residue was filtered off and washed with absolute alcohol. After centrifuging, the clear yellow solution was evaporated under reduced pressure at 40°. Dried residue, 2.63 g., 51%.

No. 81. 5.47 g. of "Stock I" were shaken with 55 cc. of absolute alcohol and glass beads. Almost all the material was shaken loose from the flask. The liquid was decanted and centrifuged in closed tubes. The

clear yellow solution was decanted and evaporated under reduced pressure at 40°. Dried residue, 2.85 g., 52.0%. A rabbit weighing 453 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 474 g. Uterus congested and enlarged. Empty body, 303 g. Dried uterus, 0.0335 g. Normal, 0.0070-0.0120 g.

Ether.—No. 82. 6.26 g. of "Stock I" were shaken with 63 cc. of ether. After decanting and centrifuging the solution the ether was evaporated under reduced pressure at 40°. Dried residue, 0.59 g., 9.4%. The residue was not tested, as it was feared that the original "Stock I" had lost its activity from long standing.

No. 83. 5.50 g. of "Stock I" were shaken with 55 cc. of ether and glass beads. The emulsion was decanted and centrifuged in closed tubes. The very cloudy supernatant liquid was decanted and evaporated under reduced pressure at 40°. Dried residue, 1.45 g., 26.4%. A rabbit weighing 440 g. was injected with 1 cc. of 2.5% emulsion. After 4 days, 488 g. Uterus much congested and enlarged. Empty body, 340 g. Dried uterus, 0.0296 g. Normal, 0.0070-0.0120 g.

Ethyl Acetate.—No. 84. 5.28 g. of "Stock I" were shaken with 53 cc. of ethyl acetate. After centrifuging, the clear yellow solution was decanted and evaporated under reduced pressure at 40°. Dried residue, 0.66 g., 12.5%.

No. 85. 4.97 g. of "Stock I" were shaken with 50 cc. of ethyl acetate. The solution was decanted from the viscous residue and centrifuged in closed tubes. The solution was decanted and evaporated under reduced pressure at 40°. Dried residue, 0.71 g., 14.3%. A rabbit weighing 450 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 497 g. Uterus congested and enlarged. Empty body, 340 g. Dried uterus, 0.0352 g. Normal, 0.0070-0.0120 g.

Carbon Disulfide.—No. 86. 5.19 g. of "Stock I" were shaken with 52 cc. of carbon disulfide and glass beads. The solution was decanted and centrifuged. The reddish-orange opalescent solution was evaporated under reduced pressure at 40°. Dried residue, 1.57 g., 30.2%.

No. 87. 4.89 g. of "Stock I" were shaken with 49 cc. of carbon disulfide and glass beads. The emulsion was decanted and centrifuged in closed tubes. The cloudy orange liquid was decanted and evaporated under reduced pressure at 40°. Dried residue, 1.65 g., 33.8%. A rabbit weighing 360 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 428 g. Uterus congested and enlarged. Empty body, 310 g. Dried uterus, 0.0330 g. Normal, 0.0070-0.0120 g.

Pyridine.—No. 88. 4.93 g. of "Stock I" were shaken with 49½ cc. of pyridine and glass beads. The emulsion was centrifuged in closed tubes. The clear brown solution was decanted and evaporated under reduced pressure at 40°. Dried residue, 1.62 g., 32.9%. A rabbit weighing 348 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 393 g. Uterus congested and enlarged. Empty body, 253 g. Dried uterus, 0.0490 g. Normal, 0.0070-0.0120 g.

Acetone.—Several attempts were made to determine the solubility of the active material in "Stock I" in acetone, but these were unsuccessful. "Stock I" on treatment with acetone became very viscous; it stuck to the flask and could not be mixed with the solvent.

No. 89. 6.16 g. of "Stock I" were shaken with 62 cc. of acetone and glass beads. The material became very gummy and did not mix at all with the acetone. After breaking the flask it was scraped out and ground with acetone in a mortar. After centrifuging the solution, it was evaporated under reduced pressure at 40°. Dried residue, 0.50 g., 8.0%. The solution was not tested, as the activity of the original "Stock I" was in question.

No. 90. 0.46 g. of "Stock II" were shaken with 5 cc. of acetone. The suspension was filtered through a dry filter and the filter washed with acetone twice. The solution was evaporated under reduced pressure at 40°. The dried residue, a clear, yellow, viscous liquid with red fluorescence, weighed 0.31 g., 67%. A rabbit weighing 608 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 652 g. Uterus congested and enlarged. Empty body, 447 g. Dried uterus, 0.0612 g. Normal, about 0.0120 g.

95% and 50% Alcohol.—No. 91. 0.52 g. of "Stock II" were shaken with 10 cc. of 95% alcohol and glass beads. The solution was decanted and the beads washed with 10 cc. of alcohol. The solutions were centrifuged. The clear yellow liquid was decanted and evaporated under reduced pressure at 40°. Dried residue, 0.45 g., 86%. This was shaken with 10 cc. of 95% alcohol. To the solution was added 9 cc. of water. A white precipitate formed. The emulsion was centrifuged. The opalescent greenish solution was pipetted off from the red liquid which had settled out and was evaporated under reduced pressure at 40°. Dried residue, 0.28 g., 54%. A rabbit weighing 491 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 523 g. Uterus much congested and enlarged. Empty body, 349 g. Dried uterus, 0.0530 g. Normal, 0.0070-0.0120 g.

No. 92. The red liquid which was precipitated from the 50% alcohol solution of the preceding experiment was dissolved in alcohol and the solution evaporated under reduced pressure at 40°. Dried residue, 0.12 g., 23%. A rabbit weighing 412 g. was injected with 1 cc. of 1% emulsion (Arnold sterilization). After 4 days, 485 g. Uterus enlarged and slightly congested. Empty body, 291 g. Dried uterus, 0.0216 g. Normal, 0.0070-0.0120 g.

60% Alcohol.—No. 93. 0.81 g. of "Stock II" were shaken with 16 cc. of 95% alcohol and glass beads, and the emulsion was centrifuged. The supernatant liquid was decanted and evaporated under reduced pressure at 40°. The residue was shaken with 16 cc. of 95% alcohol. To the solution was added 14.5 cc. of water. No separation could be obtained in this case by centrifuging, presumably because the specific gravities of the two liquid phases were the same. Globules could be seen floating in the solution. The emulsion was evaporated under reduced pressure at 40°, and the residue dissolved in 10 cc. of 94½% alcohol. 5¾ cc. of water were added and the solution was shaken and centrifuged. The supernatant pale yellow solution was pipetted off. The red liquid residue was again shaken with 10 cc. of 94½% alcohol. To the turbid solution was added 5¾ cc. of water. The emulsion was centrifuged and the upper layer pipetted off as before. The lower layer was again treated as before. The 60% alcohol solutions were evaporated under reduced pressure at 40°. Dried residue, 0.33 g., 41%. This was only partially soluble in acetone. After evaporating the acetone under reduced pressure at 40° the material was treated with ethyl acetate. Not quite all of it dissolved.

No. 94. The red liquid insoluble in 60% alcohol in the above experiment was dissolved in alcohol and chloroform and the solution evaporated under reduced pressure at 40°. Dried residue, 0.36 g., 45%. A rabbit weighing 512 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 560 g. Uterus slightly congested and slightly enlarged in spots. Empty body, 411 g. Dried uterus, 0.0140 g. Normal, 0.0080-0.0120 g.

Carbon Disulfide and Ethyl Acetate.—No. 95. 0.92 g. of "Stock II" were treated with 10 cc. of carbon disulfide. A clear solution resulted. This was evaporated under reduced pressure at 40°. The residue was shaken with 10 cc. of ethyl acetate. It partially dissolved. The addition of 10 cc. more of ethyl acetate did not effect complete solution; some viscous liquid remained insoluble. The solution was centrifuged. The clear orange liquid was decanted and evaporated under reduced pressure at 40°. A very small amount was lost by spattering. The dried

residue was a very viscous, orange liquid, weighing 0.66 g., 72%. This was shaken with 10 cc. of 95% alcohol. Not quite all dissolved.

Pyridine.—No. 96. 1.01 g. of "Stock II" were shaken with 10 cc. of pyridine. Most of the grease dissolved, giving a clear golden solution. On the addition of 5 cc. more pyridine all dissolved.

Benzin.—No. 97. 1.265 g. of "Stock II" were shaken with 13 cc. of Merck's U. S. P. benzin. The solution was centrifuged. The supernatant liquid was decanted and evaporated. Dried residue, 1.195 g., 94.5%. A rabbit weighing 443 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 465 g. Uterus congested and enlarged. Empty body, 312 g. Dried uterus, 0.0183 g. Normal, 0.0070-0.0120 g.

Glacial Acetic Acid.—No. 98. 1.05 g. of "Stock II" were shaken with 10.5 cc. of glacial acetic acid. The material dissolved to form a slightly turbid red solution with green fluorescence. This was centrifuged and the supernatant liquid decanted from the small light brown residue. The solution was evaporated under reduced pressure at 40°. It was then repeatedly evaporated with absolute alcohol until the odor of acetic acid had disappeared. Dried residue, 0.97 g., 92½%. A rabbit weighing 570 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 544 g. Uterus somewhat congested and enlarged. Empty body, 369 g. Dried uterus, 0.0285 g. Normal, 0.0070-0.0120 g.

No. 99. In another experiment, 1.235 g. of "Stock II" were shaken with 6.2 cc. of glacial acetic acid. The suspension was centrifuged. The supernatant liquid was evaporated under reduced pressure at 40°. Dried residue, 1.13 g., 91½%. A rabbit weighing 538 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 545 g. Uterus congested and enlarged. Empty body, 363 g. Dried uterus, 0.0354 g. Normal, 0.0070-0.0120 g.

No. 100. The residue insoluble in acetic acid in the above experiment was dissolved in alcohol and ether and the solution was evaporated under reduced pressure at 40°. Dried residue, 0.035 g., 3%. A rabbit weighing 518 g. was injected with 1 cc. of 0.7% emulsion. After 4 days, 557 g. Uterus slightly enlarged and congested. Empty body, 386 g. Dried uterus, 0.0188 g. Normal, 0.0080-0.0120 g.

No. 101. 1.11 g. of "Stock II" were emulsified in 11.1 cc. of glacial acetic acid. After centrifuging, the clear brown solution with greenish fluorescence was decanted into 50 cc. of water. The emulsion was then extracted with two portions of benzene, 50 cc. each, and the extract was washed with water three times, the last washing being neutral. After

centrifuging, the solution was evaporated under reduced pressure. Dried residue, 0.88 g., 79%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 440 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 458 g. A residue of unabsorbed injected material was found. Uterus congested and slightly enlarged. Empty body, 256 g. Dried uterus, 0.0147 g. Normal, 0.0070-0.0120 g.

Acetyl Chloride.—No. 102. 0.69 g. of "Stock II" were treated with 5.25 g. of acetyl chloride. All the material dissolved to form a brownish opalescent solution.

Benzene-Alcoholic Potash.—No. 103. 0.40 g. of "Stock II" were shaken with 10 cc. of benzene and 10 cc. of normal alcoholic potash. A homogeneous solution resulted. In a later, similar experiment, a small amount of red liquid settled out.

Acetic Anhydride.—No. 104. 1.285 g. of "Stock II" were treated with 25 cc. of acetic anhydride. The material did not dissolve in the cold, but on boiling dissolved completely.

E. Partition Experiments

Benzene and Water.—No. 105. 7.84 g. of "Stock I" were shaken with 78 cc. each of water and benzene. A white emulsion formed. The next day the lower, clear red solution was drawn off. The upper layer was opalescent and cloudy. After centrifuging, the clear red supernatant liquid was evaporated under reduced pressure at 40°. Dried residue, 1.25 g., 16%. A rabbit weighing 570 g. was injected with 1 cc. of 5% emulsion (Arnold sterilization). After 4 days, 508 g. Uterus enlarged and slightly congested. Empty body, 357 g. Dried uterus, 0.0233 g. Normal, 0.0070-0.0120 g.

No. 106. The aqueous layer from the preceding experiment was evaporated under reduced pressure at 40°. Dried residue, 6.19 g., 79%. A rabbit weighing 570 g. was injected with 1 cc. of 5% emulsion (Arnold sterilization). After 4 days, 575 g. Uterus normal. Empty body, 368 g. Dried uterus, 0.0099 g. Normal, 0.0070-0.0120 g.

Benzin and Water.—No. 107. Five lbs. of fresh ground placentas were treated by the ordinary method for the preparation of "Stock II" up to the point of the benzene extraction. At this point the aqueous emulsion

was extracted with two portions of Merck's U. S. P. benzin, 150 and 100 cc. respectively. The benzin solutions were centrifuged in closed tubes. The clear solution was decanted and evaporated under reduced pressure at 40°. Dried residue, 2.68 g. A rabbit weighing 417 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 380 g. Uterus normal. Empty body, 288 g. Dried uterus, 0.0093 g. Normal, 0.0070-0.0120 g.

No. 108. The aqueous layer which had been extracted with benzin was combined with the aqueous layers obtained by centrifuging the benzin solution and these were extracted with 2 portions of benzene of 150 and 100 cc. respectively. The benzene extracts were centrifuged and evaporated under reduced pressure at 40°. Dried residue, 1.00 g. A rabbit weighing 375 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 403 g. Uterus congested and much enlarged. Empty body, 243 g. Dried uterus, 0.0515 g. Normal, 0.0070-0.0120 g.

No. 109. In a repetition of the preceding experiment 10 lbs. of fresh ground placentas were treated as before. The aqueous emulsion was extracted with 5 portions of Merck's U. S. P. benzin, 100 cc. each. After centrifuging, the extract was evaporated under reduced pressure at 40°. Dried residue, 5.96 g. A rabbit weighing 695 g. was injected with 1 cc. of 5% emulsion (Arnold sterilization). After 4 days, 763 g. Uterus congested and enlarged. Empty body, 517 g. Dried uterus, 0.0346 g. Normal, about 0.0230 g.

No. 110. 1.30 g. of "Stock II" were shaken with 50 cc. of water and 50 cc. of Merck's U. S. P. benzin. The next day the aqueous layer was drawn off and evaporated under reduced pressure at 40°. Dried residue, 0.045 g., 3½%. A rabbit weighing 589 g. was injected with 1 cc. of 0.9% emulsion. After 4 days, 540 g. Uterus slightly congested and enlarged. Empty body, 378 g. Dried uterus, 0.0114 g. Normal, 0.0070-0.0120 g.

No. 111. The benzin solution from the above experiment was evaporated under reduced pressure at 40°. Dried residue, 1.21 g., 93%. A rabbit weighing 503 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 540 g. Uterus normal. Empty body, 395 g. Dried uterus, 0.0097 g. Normal, 0.0070-0.0120 g.

No. 112. 1.605 g. of "Stock II" were shaken with 50 cc. each of Merck's U. S. P. benzin and water. The aqueous layer was drawn off and evaporated under reduced pressure at 40°. Dried residue, 0.055 g., 3.4%. A rabbit weighing 323 g. was injected with 1 cc. of 1.1% emulsion. After

4 days, 292 g. Uterus somewhat congested and enlarged. Empty body, 198 g. Dried uterus, 0.0197 g. Normal, about 0.0160 g.

No. 113. The benzin solution from the above experiment was centrifuged and evaporated under reduced pressure at 40°. Dried residue, 1.51 g., 94%. This was emulsified to the volume which would have given a 2.5% emulsion of the original material. A rabbit weighing 816 g. was injected with 1 cc. (Arnold sterilization). After 4 days, 806 g. Uterus congested and greatly enlarged. Empty body, 536 g. Dried uterus, 0.0520 g. Normal, about 0.0260 g.

50% Alcohol and Benzin.—No. 114. 1.28 g. of "Stock II" were shaken with 50 cc. each of 50% alcohol and Merck's U. S. P. benzin. Two days later the still somewhat turbid alcoholic layer was drawn off. The benzin layer was evaporated under reduced pressure at 40°. Dried residue, 1.305 g. Two cc. of almond oil were added and the mixture was dissolved in a little ether, which was then evaporated. A rabbit weighing 545 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 600 g. Uterus normal. Empty body, 380 g. Dried uterus, 0.0127 g. Normal, 0.0080-0.0120 g.

No. 115. The 50% alcohol layer from the above experiment was evaporated under reduced pressure at 40°. Dried residue, 0.11 g., 8.6%. A rabbit weighing 650 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). After 4 days, 735 g. Uterus greatly congested and enlarged. Empty body, 513 g. Dried uterus, 0.0708 g. Normal, about 0.0225 g. Another rabbit weighing 355 g. was injected with 1 cc. of the emulsion. After 4 days, 350 g. No unabsorbed material was found near the site of injection. Uterus slightly congested and enlarged. Empty body, 212 g. Dried uterus, 0.0360 g. Normal, about 0.0130 g.

No. 116. 1.28 g. of "Stock II" were shaken with 50 cc. each of 50% alcohol and of Merck's U. S. P. benzin (re-distilled). The benzin layer was evaporated under reduced pressure at 40°. Dried residue, 1.035 g., 81%. A rabbit weighing 345 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 395 g. A residue of unabsorbed injected material was found. Uterus possibly slightly enlarged. Empty body, 285 g. Dried uterus 0.0118 g. Normal, 0.0070-0.0120 g.

No. 117. The 50% alcohol solution from the above experiment was evaporated under reduced pressure at 40°. Dried residue, 0.21 g., 16%. A rabbit weighing 432 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). The material was injected into the axilla and the puncture

sealed with collodion. After 4 days, 503 g. No unabsorbed injected material was found. Uterus congested and enlarged. Empty body, 330 g. Dried uterus, 0.0415 g. Normal, 0.0070-0.0120 g.

No. 118. 2.585 g. of "Stock II" were dissolved in 100 cc. of benzin. The solution was extracted with successive portions of 50% alcohol, 100 cc. each. First extract, 0.19 g., 7.4%. After adding the second extract and evaporating, the flask weighed less than before, probably due to a loss during foaming, which was especially troublesome. Third extract, 0.09 g., 3.5%.

75% Alcohol and Benzin.—No. 119. 1.15 g. of "Stock II" were shaken with 50 cc. each of 75% alcohol and Merck's U. S. P. benzin. The benzin solution was evaporated under reduced pressure at 40°. Dried residue, 0.59 g., 51.3%. The dried residue had become finely crystalline and could not be emulsified with mucilage of tragacanth, even on warming and shaking with glass beads.

No. 120. 1.155 g. of "Stock II" were shaken with 50 cc. each of Merck's U. S. P. benzin and 75% alcohol. The benzin layer was evaporated under reduced pressure at 40°. Dried residue, 0.635 g., 55%. Three cc. of almond oil were added and the whole was dissolved in ether, which was then evaporated. A rabbit weighing 600 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 613 g. Uterus normal. Empty body, 393 g. Dried uterus, 0.0093 g. Normal, 0.0080-0.0120 g.

No. 121. The 75% alcohol solution from the above experiment was evaporated under reduced pressure at 40°. Much trouble with foaming was experienced. Dried residue, 0.57 g., 49%. A rabbit weighing 617 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). The morning of the fourth day the rabbit was found dead. It weighed 488 g. An autopsy indicated a bacteremia. Uterus congested and enlarged. Empty body, 370 g. Dried uterus, 0.0346 g. Normal, 0.0070-0.0120 g.

No. 122. 5.49 g. of "Stock II" were shaken with 100 cc. each of 75% alcohol and Merck's U. S. P. benzin (redistilled). The alcoholic layer was again extracted with 100 cc. of benzin. It was then evaporated under reduced pressure at 40°. It foamed badly. Dried residue, 1.425 g., 26%.

No. 123. The second benzin extract of the above experiment was evaporated to a small volume on the steam bath and the evaporation completed under reduced pressure at 40°. Dried residue, 0.31 g., 5.6%. A

rabbit weighing 355 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 405 g. A residue of unabsorbed injected material was found. Uterus enlarged and somewhat congested. Empty body, 295 g. Dried uterus, 0.0275 g. Normal, 0.0070-0.0120 g.

No. 124. 2.22 g. of "Stock II" were shaken with 100 cc. each of 75% alcohol and Merck's U. S. P. benzin. After these were separated the alcoholic layer was extracted with three more portions of 100 cc. each of Merck's U. S. P. benzin. The alcoholic solution was evaporated under reduced pressure at 40°. Dried residue, 0.605 g., 27%. The benzin extracts were evaporated under reduced pressure at 40°. Second extract, 5%. Third extract, 0.7%. Fourth extract, 0.0%. Assuming 55% for the first extract as was found in No. 120, the total extract was 60.7%.

Benzene and 75% Alcohol.—No. 125. 50 cc. of benzene solution from which "Stock II" was usually prepared were shaken with 50 cc. of 75% alcohol. A good separation was not obtained. There were beads of liquid all through the upper layer, apparently as the result of temperature change after equilibrium had been reached. This method did not seem feasible as a method of separation.

F. Dialysis Experiments

Acetone Dialysis.—No. 126. 1.575 g. of "Stock II" were shaken with 50 cc. of acetone until all the material was shaken loose from the flask. The suspension was poured into a rubber bag which had soaked over night in ether and showed no leaks. This was suspended in a 250 cc. wide-mouth bottle in which 100 cc. of acetone were placed. The next day the dialysate was decanted and evaporated under reduced pressure at 40°. Dried residue, 0.28 g., 18%. On standing this became somewhat crystalline. One cc. of almond oil was added and the whole dissolved in ether, which was then evaporated under reduced pressure at 40°. A rabbit weighing 832 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 880 g. Uterus enlarged and greatly congested. Empty body, 622 g. Dried uterus, 0.0594 g. Normal, about 0.0370 g.

In the preparation of "Stock III" numerous samples of "Stock II" were dialysed through rubber in acetone until no more material came through. The yields in various experiments were 23%, 25.7%, 28.5%, 28.1%, and 33.1%.

Benzin Dialysis.—No. 127. 1.60 g. of "Stock II" were dissolved in 50 cc. of Merck's U. S. P. benzin and the solution was poured into a rubber bag which had soaked over night in ether and showed no leaks. One hundred cc. of benzin were placed in the outer bottle. The next day the dialysate was decanted and evaporated under reduced pressure at 40°. Dried residue, 0.245 g., 15.3%. This was beautifully crystalline. One-half a cc. of almond oil was added and the whole dissolved in ether, which was then evaporated under reduced pressure at 40°. A rabbit weighing 755 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilizafion). After 4 days, 800 g. Uterus locally congested and enlarged. Empty body, 555 g. Dried uterus, 0.0193 g. Normal, 0.0110-0.0315 g.

Ether Dialysis.—No. 128. 1.345 g. of "Stock II" were dissolved in 50 cc. of ether. The very cloudy solution was placed in a rubber bag which had soaked in ether. One hundred cc. of ether were placed in the outer bottle. The next day the filtered dialysate was evaporated under reduced pressure at 40°. Dried residue, 0.305 g., 23%. This was crystalline. A rabbit weighing 423 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). After 4 days, 473 g. An unabsorbed residue of injected material was found. The uterus was deeply congested and greatly enlarged. The tubes also showed the same effect. Empty body, 316 g. Dried uterus, 0.1317 g. Normal, 0.0070-0.0120 g.

Chloroform Dialysis.—No. 129. 1.35 g. of "Stock II" were dissolved in 5 cc. of chloroform and the solution poured into a rubber bag. The flask was rinsed with 5 cc. of chloroform and this likewise added. One hundred cc. of chloroform was placed in the outer bottle. The next day the yellow dialysate was decanted and evaporated on the steam bath after filtration. The evaporation was finished under reduced pressure at 40°. Dried residue, 0.45 g., 33%. A rabbit weighing 435 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). After 4 days, 500 g. Some residue of unabsorbed injected material was found. Uterus congested and enlarged. Empty body, 395 g. Dried uterus, 0.0667 g. Normal, 0.0080-0.0120 g.

Carbon Tetrachloride Dialysis.—No. 130. 1.41 g. of "Stock II" were dissolved in 5 cc. of carbon tetrachloride and the solution was poured into a rubber bag which, after soaking in ether, had shown no leaks. The flask was rinsed with 5 cc. of carbon tetrachloride and this added likewise. One hundred cc. of carbon tetrachloride were placed in the outer bottle. The next day the colorless dialysate was decanted and evaporated under reduced pressure at 40°. Dried residue, 0.22 g., 15½%. The ampoule containing the emulsion broke during sterilization.

No. 131. 1.275 g. of "Stock II" were dissolved in 5 cc. of carbon tetrachloride and poured into a rubber bag soaked in ether and found tight. The flask was rinsed with two portions of 5 cc. each of carbon tetrachloride and these were added likewise. One hundred and fifty cc. of carbon tetrachloride were placed in the outer flask. Glass beads were added to the rubber bag to prevent its floating on the liquid. Two days later the dialysate was decanted. Owing to the softening of the rubber by the carbon tetrachloride, it was impossible to recover all the dialysate. Probably 75 or 80% were decanted, about 2 cc. of the inner liquid being mixed with it. After filtration the nearly colorless dialysate was evaporated under reduced pressure at 40°. The dried residue formed crystalline rosettes and weighed 0.22 g., 17%. A rabbit weighing 565 g. was injected with 1 cc. of 2.39% emulsion (autoclaved). After 4 days, 607 g. An unabsorbed residue of injected material was found. Uterus somewhat enlarged and congested. Empty body, 362 g. Dried uterus, 0.0227 g. Normal, 0.0080-0.0120 g.

Benzene Dialysis.—No. 132. Fifty cc. of benzene solution which on evaporation would have yielded 1.28 g. of "Stock II" were placed in a rubber bag soaked in benzene. One hundred cc. of benzene were placed in the outer bottle. The next day the dialysate was decanted and evaporated under reduced pressure at 40°. Dried residue, 0.785 g., 61%. A rabbit weighing 475 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). After 4 days, 335 g. Uterus possibly enlarged. Empty body, 250 g. Dried uterus, 0.0204 g. Normal, 0.0070-0.0120 g.

No. 133. The solution inside the bag was evaporated under reduced pressure at 40°. Dried residue, 0.485 g., 38%. A rabbit weighing 640 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). After 4 days, 705 g. Uterus congested and enlarged. Empty body, 480 g. Dried uterus, 0.0722 g. Normal, about 0.0160 g.

No. 134. Fifty cc. of benzene solution which on evaporation would have yielded 1.37 g. of "Stock II" were placed in a rubber bag previously soaked in ether and found tight. One hundred cc. of benzene were placed in the outer bottle. The next day the dialysate was evaporated under reduced pressure at 40°. Dried residue, 0.28 g., 20%. A rabbit weighing 505 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). After 4 days, 572 g. Uterus deeply congested and greatly enlarged. Empty body, 396 g. Dried uterus, 0.1266 g. Normal, 0.0080-0.0120 g.

No. 135. Fifty cc. of benzene solution which on evaporation would have yielded 1.165 g. of "Stock II" were placed in a rubber bag soaked in ether and found tight. One hundred cc. of benzene were placed in the

outer bottle. Two days later the dialysate was decanted and evaporated under reduced pressure at 40°. Dried residue, 0.23 g., 20%. A rabbit weighing 670 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). The material was injected into the axilla and the puncture was sealed with collodion. After 4 days, 716 g. A residue of unabsorbed injected material was found close to the puncture. Uterus greatly congested and enlarged. Empty body, 451 g. Dried uterus, 0.0906 g. Normal, about 0.0122 g.

50% Alcohol Dialysis.—No. 136. 1.735 g. of "Stock II" were shaken with 50 cc. of 50% alcohol. The suspension was placed in a collodion bag (prepared by coating a large test-tube with collodion, letting it evaporate until no longer sticky, and then filling with water). In the outer bottle were placed 100 cc. of 50% alcohol. The next day the dialysate was decanted and evaporated under reduced pressure at 40°. Dried residue, 0.005 g., 0.3%.

62½% Alcohol Dialysis.—No. 137. 1.28 g. of "Stock II" were shaken with 50 cc. of 62½% alcohol. The emulsion was placed in a collodion bag which had been soaked in 62½% alcohol. In the outer bottle were placed 100 cc. of 62½% alcohol. Two days later the dialysate was decanted and evaporated under reduced pressure at 40°. Dried residue, 0.305 g., 24%. A rabbit weighing 480 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). After 4 days, 580 g. Uterus congested and enlarged. Empty body, 393 g. Dried uterus, 0.0380 g. Normal, 0.0080-0.0120 g.

No. 138. 4.20 g. of "Stock II" were emulsified with 60 cc. of 62½% alcohol. The liquid was dialysed through collodion into 11 successive portions of 100 cc. of 62½% alcohol. At this point dialysis was not yet complete. Dialysate, 2.405 g., 57%.

75% Alcohol Dialysis.—No. 139. 1.635 g. of "Stock II" were shaken with 50 cc. of 75% alcohol. The emulsion was placed in a collodion bag which had been soaked in 75% alcohol. Two days later the pale yellow dialysate was decanted and evaporated under reduced pressure at 40°. Dried residue, 0.48 g., 29%. The material was a yellow grease. A rabbit weighing 690 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 707 g. Uterus congested and enlarged. Empty body, 460 g. Dried uterus, 0.0439 g. Normal, about 0.0135 g.

62½% Alcohol Dialysis of Cholesterol.—No. 140. Several tenths of a g. of solid pure cholesterol were placed in a collodion bag with 10 cc. of 62½% alcohol. Two hundred cc. of 62½% alcohol were placed in

the outer bottle and the bottle was placed in a water bath at 40°. The next day the dialysate was decanted and evaporated under reduced pressure at 40°. Residue, 0.07 g. This gave the Liebermann-Burchard test for cholesterol.

Later Dialysates in "Stock III" Preparation.—No. 141. The third, fourth, and fifth 62½% alcohol dialysates of the acetone dialysate of 4.20 g. of "Stock II" were evaporated under reduced pressure at 40°. Residue, 0.21 g., 5.0%. A rabbit weighing 425 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 466 g. A residue of unabsorbed injected material was found. Uterus congested and much enlarged. Empty body, 283 g. Dried uterus, 0.0662 g. Normal, 0.0070-0.0120 g.

G. Effect of Conditions and Reagents

Arnold Sterilization of Emulsion.—No. 142. A rabbit weighing 405 g. was injected with 1 cc. of 2.5% emulsion of "Stock I" which had been prepared aseptically and then sterilized by the Arnold method. After 4 days, 442 g. Empty body, 276 g. Dried uterus, 0.0310 g. Normal, 0.0070-0.0120 g. For tests of the same emulsion, not sterilized by the Arnold method, see No. 59.

Vacuum Heating.—No. 143. 0.47 g. of "Stock II" were sealed in an evacuated glass tube which was then heated in the steam bath for 14 days. The color of the substance changed from yellow to dark brown. The tube was then opened and washed out with chloroform. Not all of the material dissolved. The solution was evaporated under reduced pressure at 40°. Dried residue, 0.44 g. A rabbit weighing 373 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). This animal died 3½ days after injection, apparently from a bacteremia. Its weight was 261 g. The weighing of the empty body was omitted, but assuming the digestive tract to have been empty and to have weighed 50 g., the empty body would have weighed 211 g. Dried uterus, 0.0409 g. Normal, about 0.0130 g.

Heating of Alcoholic Solution.—No. 144. 0.705 g. of "Stock II" were extracted with 10 cc. of 95% alcohol. A portion of the solution was sealed in an ampoule and heated in the steam bath for 4 days. On opening the ampoule the solution was found to have a very disagreeable odor, reminding one of that of burnt meat. The solution was evaporated under

reduced pressure at 40°. Dried residue, 0.44 g. From No. 91, 86% of "Stock II" is alcohol-soluble. Accordingly the amount of "Stock II" corresponding to the dried residue was 0.51 g. The dried residue was emulsified to the volume which would have given a strength of 2.5%, had the original 0.51 g. of "Stock II" been diluted to that volume. A rabbit of 680 g. was injected with 1 cc. of emulsion (Arnold sterilization). After 4 days, 770 g. Uterus congested and enlarged. Empty body, 527 g. Dried uterus, 0.0505 g. Normal, about 0.0250 g.

Autoclaving of Emulsion.—No. 145. A 2.5% emulsion of "Stock II" was autoclaved at 15 to 20 lbs. steam pressure for thirty minutes. A rabbit weighing 754 g. was injected with 1 cc. After 4 days, 930 g. Uterus much congested and enlarged. Empty body, 715 g. Dried uterus, 0.0636 g. Normal, about 0.0440 g.

No. 146. Some of the same emulsion was sterilized by the Arnold method and a rabbit weighing 425 g. was injected with 1 cc. After 4 days, 375 g. Uterus congested and enlarged. Empty body, 272 g. Dried uterus, 0.0352 g. Normal, 0.0070-0.0120 g.

Heating of Aqueous Emulsion.—No. 147. A 10% emulsion of "Stock II" in pure water was sealed in an ampoule and heated in the steam bath for 4 days. The emulsion was evaporated under reduced pressure at 40°. Dried residue, 0.405 g. A rabbit weighing 578 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 675 g. Uterus congested and enlarged. Empty body, 445 g. Dried uterus, 0.0464 g. Normal, about 0.0120 g.

Heating in Air.—No. 148. 0.775 g. of "Stock II" were dissolved in ether and evaporated in a porcelain dish on the steam bath. The material was left on the bath for one week, being dissolved in ether and evaporated each day in order to expose fresh surface to oxidation. The material darkened very noticeably in one day and at the end of the period was a dark brown tar, of which a large portion was insoluble in ether. It was dissolved in chloroform, which was then evaporated on the steam bath. Dried residue, 0.70 g. This was emulsified to the volume which would have given a 2.5% emulsion of the original material. A rabbit weighing 492 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 500 g. A residue of unabsorbed injected material was found. Uterus normal. Empty body, 330 g. Dried uterus, 0.0084 g. Normal, 0.0070-0.0120 g.

Stability of Emulsion.—No. 149. A rabbit was injected with 1 cc.

of 2.38% emulsion of "Stock I" (prepared aseptically). After 4 days it was killed. Empty body, 435 g. Dried uterus, 0.0391 g. Normal, about 0.0110 g.

No. 150. The same emulsion was preserved in a bottle stoppered with cotton for five weeks. A rabbit weighing 472 g. was injected with 1 cc. After 4 days, 534 g. Empty body, 334 g. Dried uterus, 0.0121 g. Normal, 0.0070-0.0120 g.

Another rabbit weighing 476 g. was injected with 1 cc. After 4 days, 531 g. Empty body, 339 g. Dried uterus, 0.0179 g. Normal, 0.0070-0.0120 g.

Stability in Alcoholic Solution.—No. 151. A rabbit weighing 414 g. was injected with 1 cc. of 4.76% emulsion of "Stock I" (prepared aseptically). After 4 days, 436 g. Empty body, 315 g. Uterus deeply congested. Dried uterus, 0.0528 g. Normal, 0.0070-0.0120 g.

No. 152. "Stock I" was prepared from the same alcoholic solution, after it had stood in the laboratory about six weeks longer. A rabbit weighing 635 g. was injected with 1 cc. of 5% emulsion (prepared aseptically). After 4 days, 665 g. Uterus slightly congested and slightly enlarged in spots. Empty body, 458 g. Dried uterus, 0.0182 g. Normal, about 0.0130 g.

Stability in Benzene Solution.—No. 153. A rabbit weighing 691 g. was injected with 1 cc. of 2.5% emulsion of "Stock II" (Arnold sterilization). After 4 days, 653 g. Uterus congested and much enlarged. Empty body, 486 g. Dried uterus, 0.0587 g. Normal, 0.0170 g.

No. 154. Five weeks later another sample of "Stock II" was prepared from the same benzene solution as the previous one. A rabbit weighing 518 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 561 g. Uterus congested and greatly enlarged. Empty body, 384 g. Dried uterus, 0.0964 g. Normal, 0.0070-0.0120 g.

Stability in Solid Form.—No. 155. Twenty-nine lbs. of fresh ground placentas were stirred with 15 l. of 95% alcohol. Three days later, the juice was pressed out, filtered, and evaporated under reduced pressure to a small volume. The emulsion was then extracted with alcohol and ether, the extract was evaporated under reduced pressure, and the residue kept in a corked flask. Two and a half years later, this was dissolved in 95% alcohol, evaporated under reduced pressure at 40°, and extracted with benzene as in the ordinary preparation of "Stock II." A rabbit weighing 665 g. was injected with 1 cc. of 2.5% emulsion (Arnold

sterilization). After 4 days, 770 g. Uterus congested and enlarged. Empty body, 572 g. Dried uterus, 0.0460 g. Normal, about 0.0320 g.

Aqueous Sulfuric Acid—Four Days.—No. 156. 0.92 g. of "Stock II" were dissolved in carbon disulfide and evaporated, then extracted with ethyl acetate, as solubility experiments. The dried residue after evaporation of the ethyl acetate was shaken with 25 cc. of 2N sulfuric acid and glass beads. Four days later the emulsion was decanted from the beads and these were washed with water. The emulsion was extracted twice with benzene, and the extract washed with water. After centrifuging, the clear solution was evaporated under reduced pressure at 40°. Dried residue, 0.59 g., 64%. The residue was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 592 g. was injected with 1 cc. of the emulsion (Arnold sterilization). After 4 days, 520 g. The tip of one horn of the uterus was congested and enlarged. Empty body, 370 g. Dried uterus, 0.0079 g. Normal, 0.0070-0.0120 g.

Aqueous Sulfuric Acid—One Day.—No. 157. 0.955 g. of "Stock II" were shaken with 25 cc. of 2N sulfuric acid and glass beads. The next day the emulsion was very viscous. It was decanted from the glass beads and these were washed with water. The emulsion was extracted three times with benzene and the combined extracts were washed five times with water, the last washing being neutral to litmus. After centrifuging the extract it was evaporated under reduced pressure at 40°. Dried residue, 0.585 g., 61½%. The material was liquid at 40°, but became solid on standing at room temperature. It was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 660 g. was injected with 1 cc. of the emulsion (Arnold sterilization). After 4 days, 616 g. Uterus enlarged, but without congestion. Empty body, 442 g. Dried uterus, 0.0202 g. Normal, about 0.0115 g.

No. 158. 1.315 g. of "Stock II" were shaken with 25 cc. of 2N sulfuric acid and glass beads. The next day 75 cc. of water and 100 cc. of benzene were added to the viscous emulsion. After shaking well, the emulsion was decanted from the beads. These were washed with two portions of benzene, 10 cc. each. After settling, the aqueous layer and the emulsion were drawn off from the benzene layer and extracted with two portions of 50 cc. of benzene each. The combined benzene extracts were washed with water four times, the last washing being neutral. After centrifuging, the clear solution was evaporated under reduced pressure at 40°. Dried residue, 0.955 g., 73%. This was emulsified to the volume which would

have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 420 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla, and the puncture sealed with collodion. After 4 days, 483 g. A residue of unabsorbed injected material was found. The uterus showed no congestion but possibly slight enlargement. Empty body, 318 g. Dried uterus, 0.0120 g. Normal, 0.0070-0.0120 g.

Aqueous Sulfuric Acid—One Hour.—No. 159. 0.765 g. of "Stock II" were shaken with 25 cc. of 2N sulfuric acid and glass beads. After 45 minutes all had emulsified. 75 cc. of water were added and the emulsion was decanted from the beads. Six minutes later the emulsion was shaken with 100 cc. of benzene. After about two hours' standing the aqueous layer and emulsion were drawn off. These were re-extracted with benzene twice. The combined benzene extracts were washed with water six times, the last washing being neutral. After centrifuging, the benzene solution was evaporated under reduced pressure at 40°. Dried residue, 0.575 g., 75%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 530 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla, and the puncture sealed with collodion. After 4 days, 575 g. A residue of unabsorbed injected material was found. The uterus was enlarged and slightly congested. Empty body, 398 g. Dried uterus, 0.0420 g. Normal, 0.0080-0.0120 g.

No. 160. To the acid solution and washings from the above experiment, 2N sodium hydroxide was added until the solution was alkaline to Congo red. It was then extracted with two portions of benzene, 100 cc. each. The extracts were washed with water once, and evaporated under reduced pressure at 40°. The dried residue was too small to be weighable.

Sodium Ethoxide.—No. 161. 2.25 g. of "Stock II" were dissolved in 50 cc. of ether. A solution of 2.5 g. of sodium in 50 cc. of absolute alcohol was added and the mixture was well shaken. The next day the soaps were filtered off and washed with ether. The saponification mixture had an odor recalling that of pyridine. The ethereal filtrate was red by transmitted, and green by reflected light. It was washed once with water. This washing was extracted with ether twice and the ether extracts were added to the original one. The combined ether extracts were washed twice with water, three times with dilute sodium hydroxide, and twice more with water. The solution was evaporated under reduced pressure at 40°. Dried residue, 0.35 g., 13.5%. A rabbit weighing 375 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 450 g.

Uterus congested and somewhat enlarged. Empty body, 278 g. Dried uterus, 0.0207 g. Normal, 0.0070-0.0120 g.

Cold Alcoholic Potash—Modified Henriques[36] Method.—No. 162. 0.40 g. of "Stock II" were dissolved in 10 cc. of benzene. To this were added 10 cc. of normal alcoholic potash. A homogeneous solution resulted. After two days, water and more benzene were added. After drawing off the aqueous layer it was extracted twice more with benzene. The combined extracts were washed with water three times. These washings were extracted with benzene and the extract, after washing once with water, was added to the former one. After centrifuging, the solution was evaporated under reduced pressure at 40°. Dried residue, 0.045 g., 11%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 610 g. was injected with 1 cc. of the emulsion (Arnold sterilization). After 4 days, 555 g. Uterus normal. Empty body, 420 g. Dried uterus, 0.0080 g. Normal, 0.0080-0.0120 g.

No. 163. 25 cc. of benzene solution which on evaporation would have yielded 0.58 g. of "Stock II" were shaken with 25 cc. of normal alcoholic potash. The solution became a deeper red. The next day some red oil was noted on the bottom of the flask. The mixture was shaken with 100 cc. each of water and benzene. The separation was very poor. After drawing off the aqueous layer, it was extracted with two more portions of benzene of 100 cc. each. The combined benzene extracts were let stand over night and the aqueous layer which had settled out was drawn off. This was extracted with 25 cc. of benzene and the extract combined with the previous ones. The benzene and emulsion layers were washed with water three times. The third washing was neutral. After centrifuging, the clear benzene solution was evaporated under reduced pressure at 40°. Dried residue, 0.145 g., 25%. Several drops of almond oil were added and the mixture was dissolved in ether. After this had been evaporated under reduced pressure the residue was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 395 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 463 g. A residue of unabsorbed injected material was found. Uterus slightly congested and enlarged. Empty body, 312 g. Dried uterus, 0.0137 g. Normal, 0.0070-0.0120 g. The higher yield of unsaponified material in this experiment makes it appear probable that some of the active material escaped hydrolysis by being carried down with the insoluble red oil.

Aqueous Sodium Hydroxide.—No. 164. 0.93 g. of "Stock II" were shaken with 25 cc. of 2N sodium hydroxide and glass beads. A soapy, heavy emulsion was formed. The next day, after adding water, it was extracted with benzene three times. The extract was washed with water three times. The last washing was neutral. The benzene layer was centrifuged and evaporated under reduced pressure at 40°. Dried residue, 0.03 g., 3%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 670 g. was injected with 1 cc. of emulsion (Arnold sterilization). After 4 days, 655 g. Uterus slightly congested and enlarged locally. Empty body, 455 g. Dried uterus, 0.0212 g. Normal, about 0.0128 g.

No. 165. 1.05 g. of "Stock II" were shaken with 25 cc. of 2N sodium hydroxide and glass beads. The next day the solid curd was treated with 75 cc. of water and 100 cc. of benzene. The emulsion was decanted from the beads and these were washed with 10 cc. of benzene. The aqueous layer and emulsion were drawn off and extracted with two portions of 100 cc. of benzene each. The combined extracts were washed with water twice. The second washing was neutral. The benzene solution was evaporated under reduced pressure at 40°. Dried residue, 0.035 g., 3.5%. A few drops of almond oil were added and the mixture was dissolved in ether which was then evaporated under reduced pressure at 40°. The residue was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 572 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla, and the puncture sealed with collodion. After 4 days, 680 g. No residue of unabsorbed injected material was found. Uterus enlarged, but not congested. Empty body, 512 g. Dried uterus 0.0698 g. Normal, about 0.0220 g.

No. 166. 1.22 g. of "Stock II" were emulsified with 25 cc. of 2N aqueous sodium hydroxide and glass beads. The next day 75 cc. of water and 100 cc. of benzene were added. The separation was very poor. After centrifuging the mixture the aqueous and emulsion layers were extracted in a separatory funnel with six portions of benzene of 100 cc. each. The combined benzene and emulsion layers were centrifuged and the emulsion layer was again shaken with 100 cc. of benzene and centrifuged. The combined benzene extracts were washed once with water, the washing being neutral. The solution was evaporated under reduced pressure at 40°. Dried residue, 0.105 g., 8.6%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 355 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla and the puncture

sealed with collodion. After 4 days, 357 g. A very small residue of unabsorbed injected material was found. Uterus normal. Empty body, 222 g. Dried uterus, 0.0093 g. Normal, 0.0070-0.0120 g.

No. 167. The extracted aqueous layer from the above experiment was acidified with 2N sulfuric acid, made alkaline with 2N potassium hydroxide, and then made just acid with 2N sulfuric acid. It was then extracted with 100 cc. of ether and two portions of benzene, 100 cc. each. The extract was washed with water three times and centrifuged; it was then evaporated under reduced pressure at 40°. Dried residue, 0.66 g., 54%. A rabbit weighing 700 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 714 g. A residue of unabsorbed injected material was found. The uterus was possibly slightly enlarged, but not appreciably congested, although there was a slight injection of the vessels of the broad ligament and uterus. Empty body, 524 g. Dried uterus, 0.0189 g. Normal, 0.0120 g.-about 0.0230 g.

Barium Hydroxide—Modified Smith and Mair[37] Procedure.—No. 168. 1.77 g. of "Stock II" were dissolved in ether and the solution was diluted to 89 cc. To 50 cc. of this solution, containing 1.00 g. of "Stock II," were added 50 cc. of methyl alcohol. After the addition of a little talc, 28.4 cc. of 0.3525 N barium hydroxide in 80% methyl alcohol (equivalent to 100 cc. of N/10) were added. After boiling down on the steam bath to 50 cc., the solution was boiled with reflux condenser for 4¼ hours. It was then made acid to phenolphthalein with 0.5 cc. of glacial acetic acid and evaporated under reduced pressure at 40°. The residue was shaken with 25 cc. each of water and benzene, and the emulsion centrifuged. After decanting the clear solution, the emulsion was again shaken with 25 cc. of benzene and centrifuged. The combined extracts were evaporated under reduced pressure at 40°. Dried residue, 0.72 g., 72%. This was emulsified to the volume which would have given a 1.3% emulsion of the original "Stock II." A rabbit weighing 537 g. was injected with 1 cc. of emulsion (autoclaved). After 4 days, 605 g. Uterus normal. Empty body, 337 g. Dried uterus, 0.0114 g. Normal, 0.0070-0.0120 g.

No. 169. 1.165 g. of "Stock II" were dissolved in ether and the solution was diluted to 58 cc. To 50 cc. of this solution (containing 1.00 g. of "Stock II") were added 50 cc. of methyl alcohol. After adding a few grains of talc, 28.4 cc. of 0.3525 N barium hydroxide in 80% methyl alcohol were added (equivalent to 100 cc. of N/10). The solution was

boiled down on the steam bath to 50 cc. and then boiled with reflux condenser 4¾ hours. After the addition of phenolphthalein it was acidified with 0.44 cc. of glacial acetic acid and evaporated under reduced pressure at 40°. The residue was shaken with 50 cc. each of water and benzene. The emulsion was centrifuged and the benzene layer decanted. The aqueous layer was again shaken with 50 cc. of benzene. The emulsion was centrifuged and the benzene layer combined with the former one. The solution was evaporated under reduced pressure at 40°. Dried residue, 0.72 g., 72%. After the addition of 1 cc. of almond oil the mixture was dissolved in ether, and this was then evaporated under reduced pressure at 40°. The residue was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 490 g. was injected with 1 cc. of emulsion (autoclaved). The material was injected into the axilla, and the puncture sealed with collodion. After 4 days, 535 g. A residue of unabsorbed injected material was found. Uterus greatly congested and enlarged. Empty body, 360 g. Dried uterus, 0.0619 g. Normal, 0.0070-0.0120 g.

No. 170. 1.085 g. of "Stock II" were dissolved in ether and the solution was diluted to 54 cc. To 50 cc. of the solution (containing 1.00 g. of "Stock II") were added 50 cc. of methyl alcohol, 27.9 cc. of 0.359 N barium hydroxide in 80% methyl alcohol (equivalent to 100 cc. of N/10), and a few grains of talc. After boiling down to 50 cc. on the steam bath, the solution was boiled 4¼ hours with reflux condenser. Phenolphthalein was then added and the mixture acidified with glacial acetic acid, 0.40 cc. being required. It was then evaporated under reduced pressure at 40°. After shaking with 50 cc. each of benzene and water, the emulsion was centrifuged. The aqueous emulsion layer was similarly extracted with three portions of benzene, 50 cc. each. The benzene extracts were washed with water twice, centrifuged, and evaporated under reduced pressure at 40°. Dried residue, 0.715 g., 71.5%. One cc. of almond oil was added and the mixture dissolved in ether, which was then evaporated on the steam bath. The residue was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 379 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 440 g. A residue of unabsorbed injected material was found. Uterus congested and enlarged. Empty body, 313 g. Dried uterus, 0.0587 g. Normal, 0.0070-0.0120 g.

Soy-bean Lipase.—No. 171. 1.075 g. of "Stock II" were shaken with 10 cc. of water and 1 g. of dried ether-extracted soy-bean flour. The

neck of the flask was washed down with 17 cc. of water. After adding a little toluene the flask was kept in a thermostat at about 37° for a week, shaking occasionally with glass beads to emulsify the grease which separated. The material was then extracted with three portions of benzene, 100 cc. each. After centrifuging, the solution was evaporated under reduced pressure at 40°. Dried residue, 0.99 g., 92%. The residue was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 655 g. was injected with 1 cc. of the emulsion (Arnold sterilization). After 4 days, 800 g. Uterus congested and enlarged. Empty body, 595 g. Dried uterus, 0.0414 g. Normal, 0.0110 g.-about 0.0347 g.

Aqueous Sodium Carbonate.—No. 172. 1.01 g. of "Stock II" after having been dissolved in pyridine and evaporated to dryness in a solubility experiment were shaken with 25 cc. of 10% sodium carbonate and glass beads. The next day the emulsion was diluted with water and extracted with benzene twice. After centrifuging, the slightly cloudy solution was evaporated under reduced pressure at 40°. Dried residue, 0.10 g., 10%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 475 g. was injected with 1 cc. of the emulsion (Arnold sterilization). After 4 days, 420 g. Uterus enlarged and deeply congested. Empty body, 346 g. Dried uterus, 0.0393 g. Normal, 0.0070-0.0120 g.

No. 173. 1.585 g. of "Stock II" were shaken with 25 cc. of 10% sodium carbonate, warming. The next day the emulsion was extracted with three portions of benzene, 100 cc. each. After centrifuging, the clear solution was evaporated under reduced pressure at 40°. Dried residue, 1.29 g., 81.4%.

Bromine.—In the early bromination experiments, the chloroform solution was simply evaporated. The residue was very sticky, and it was found impossible to get it to emulsify until after purifying it further.

No. 174. 1.06 g. of "Stock II" were dissolved in chloroform. Bromine was added until the red color persisted, then several drops more. The next day the solution was evaporated under reduced pressure at 40°. The residue was taken up in chloroform and the solution again evaporated. This treatment was repeated. The material still had a slight odor recalling that of bromine. It was emulsified with absolute alcohol and glass beads and the emulsion was centrifuged. The clear yellow solution was decanted from the white residue and evaporated under reduced pressure at 40°. Dried residue, 1.485 g. This was emulsified to the volume which

would have given a 2.5% emulsion of the original "Stock II." The emulsification was not very successful. A rabbit weighing 783 g. was injected with 1 cc. of the emulsion (Arnold sterilization). After 4 days, 795 g. Uterus normal. Empty body, 600 g. Dried uterus, 0.0108 g. Normal, 0.0110-0.0350 g.

No. 175. 0.875 g. of "Stock II" were dissolved in chloroform and an excess of bromine added. The next day the solution was evaporated under reduced pressure at 40°. The residue was extracted with carbon tetrachloride and the solution evaporated under reduced pressure at 40°. The residue still had a slight odor recalling that of bromine. It was extracted with acetone and the solution centrifuged. The clear supernatant liquid was evaporated under reduced pressure at 40°. The residue was extracted with three portions of 95% alcohol, 10 cc. each. The solution was evaporated under reduced pressure at 40°. Dried residue, 0.995 g. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 427 g. was injected with 1 cc. of the emulsion (Arnold sterilization). After 4 days, 430 g. Uterus slightly congested and enlarged. Empty body, 312 g. Dried uterus, 0.0165 g. Normal, 0.0070-0.0120 g.

No. 176. 2.22 g. of "Stock II" were emulsified in 100 cc. of 75% alcohol. This was extracted with four portions of Merck's U. S. P. benzin, 100 cc. each, as a partition experiment. The alcoholic solution was evaporated under reduced pressure at 40°. Dried residue, 0.605 g., 27%. To this was added 10 cc. of chloroform and an excess of bromine. The first portion of bromine was seen to be decolorized. No vapors of hydrobromic acid were detected. The next day the solution was centrifuged and the supernatant liquid evaporated under reduced pressure at 40°. Dried residue, 0.95 g. A rabbit weighing 590 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). After 4 days, 710 g. Uterus somewhat enlarged, but not congested, except the tip of one horn. Empty body, 472 g. Dried uterus, 0.0204 g. Normal, 0.0115-about 0.0150 g.

No. 177. 0.27 g. of "Stock III" were dissolved in 10 cc. of chloroform. 0.5 cc. of a solution of 1 cc. of bromine in 9 cc. of chloroform were then added slowly. The first few drops were decolorized. The mixture was then heated to boiling on the steam bath. On blowing across the mouth of the flask a slight cloud was observed, which, however, did not redden litmus paper. The remainder of the bromine solution was then added and the mixture permitted to stand over night, when it was evaporated under reduced pressure at 40°. Dried residue, 0.50 g. The residue was then emulsified to the volume which would have given a 2.5%

emulsion of the original "Stock III." A rabbit weighing 580 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 614 g. A slight amount of unabsorbed injected material was found. Uterus congested and enlarged. Empty body, 444 g. Dried uterus, 0.0424 g. Normal, about 0.0120 g.

No. 178. 0.555 g. of "Stock III" were dissolved in 10 cc. of chloroform, and the solution, after the addition of 5 cc. of bromine, was permitted to stand 5 days. It was then diluted to 60 cc.. Thirty cc. were used in No. 179. The other 30 cc. were evaporated under reduced pressure at 40°. Residue, 0.555 g. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock III." A rabbit weighing 545 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 563 g. The residue of unabsorbed injected material was quite small, and well spread out. Uterus not congested, possibly slightly enlarged. The vessels of the broad ligament were slightly injected. Empty body, 372 g. Dried uterus, 0.0148 g. Normal, 0.0080-0.0120 g.

Reduction of Brominated "Stock III" with Zinc and Glacial Acetic Acid.—No. 179. Thirty cc. (one-half) of the chloroform solution of the brominated "Stock III" of the previous experiment were evaporated under reduced pressure at 40°. Residue, 0.56 g. This was dissolved in 25 cc. of glacial acetic acid. After the addition of 5 g. of zinc dust, the mixture was boiled gently for 5 hours, shaking frequently. Fifty cc. each of water and benzene were then added. After shaking well, the emulsion was decanted from the zinc, which was washed with 25 cc. each of water and benzene. After combining the solutions, the aqueous layer was drawn off and extracted with two portions of benzene, 50 cc. each. The combined extracts were washed with four portions of water, 100 cc. each; the last washing was neutral. After centrifuging, the benzene solution was evaporated under reduced pressure at 40°. Residue, 0.24 g., 86%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock III." A rabbit weighing 632 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 630 g. A residue of unabsorbed injected material was found. Uterus slightly congested and enlarged. Empty body, 437 g. Dried uterus, 0.0153 g. Normal, 0.0080-0.0120 g.

Alkaline Permanganate.—No. 180. 1.25 g. of "Stock II" were

extracted with 10 cc. of 95% alcohol. The solution was decanted into about 150 cc. of water. This was evaporated under reduced pressure at 40° to a volume of 100 cc. Eleven g. of sodium carbonate were dissolved in the solution and 40 cc. of 5% potassium permanganate solution added slowly. The solution showed a purple color. It was extracted twice with benzene. After centrifuging, the solution was evaporated under reduced pressure at 40°. Dried residue, 0.01 g., 0.1%. The residue contained small crystalline rosettes. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 357 g. was injected with 1 cc. of emulsion (Arnold sterilization). After 4 days, 331 g. Uterus perhaps slightly enlarged. Empty body, 220 g. Dried uterus, 0.0120 g. Normal, about 0.0117 g.

No. 181. 1.15 g. of "Stock II" were shaken with 10 cc. of ether. To the opalescent solution, 100 cc. of water were added; the ether was then evaporated under reduced pressure at 40°. Eleven grams of sodium carbonate were dissolved in the emulsion, and 5% potassium permanganate was stirred in slowly. Forty cc. gave a red color, permanent for 15 minutes. After shaking with 100 cc. benzene, the emulsion was centrifuged. The aqueous layer, from which the manganese dioxide had been precipitated by the centrifuging, was extracted with two portions of benzene, 100 cc. each. The combined benzene extracts were washed with three portions of water, 100 cc. each. After centrifuging, the solution was evaporated under reduced pressure at 40°. Residue, 0.045 g., 3.9%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 660 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 745 g. Only a trace of residue of unabsorbed injected material was found. Uterus slightly enlarged and very slightly congested. Empty body, 505 g. Dried uterus, 0.0177 g. Normal, 0.0130-about 0.0220 g.

Zinc and Glacial Acetic Acid.—No. 182. 0.835 g. of "Stock II" were dissolved in 10 cc. of glacial acetic acid. Five g. of zinc powder were added and the mixture was shaken well. It was permitted to stand for one day at room temperature with shaking at intervals. The zinc was then filtered off and washed with glacial acetic acid. An excess of water was added to the solution and it was extracted twice with benzene. The extract was washed three times with water, once with 10% sodium carbonate solution, and once more with water. After centrifuging, the solution was evaporated under reduced pressure at 40°. Dried residue, 0.49 g., 59%. A rabbit weighing 660 g. was injected with 1 cc of 2.5% emulsion

(Arnold sterilization). After 4 days, 726 g. Uterus enlarged and somewhat congested. Empty body, 470 g. Dried uterus, 0.0550 g. Normal, 0.0115-0.0150 g.

No. 183. 0.745 g. of "Stock II" were dissolved in 25 cc. of glacial acetic acid. Five g. of zinc dust were added gradually with shaking. The mixture was boiled gently for four hours, shaking frequently. Fifty cc. each of water and benzene were then added and the mixture was well shaken. After settling, the emulsion was decanted from the zinc powder into a separatory funnel. The zinc powder was washed with 25 cc. of water and 25 cc. of benzene. After drawing off the aqueous layer the benzene layer was washed with water about five times, the last washing being neutral. After centrifuging, the solution was evaporated under reduced pressure at 40°. Dried residue, 0.615 g., 82½%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 405 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 448 g. A residue of unabsorbed injected material was found. Uterus greatly congested and enlarged. Empty body, 322 g. Dried uterus, 0.1135 g. Normal, 0.0070-0.0120 g.

Nitrous Acid.—No. 184. 4.91 g. of "Stock II" were shaken with 49 cc. of glacial acetic acid and the solution was poured into a solution of 30 g. of sodium nitrite in 100 cc. of water. Some of the material was lost by foaming over. The solution was neutralized with sodium carbonate solution and extracted with 100 cc. of benzene. The extract was evaporated under reduced pressure at 40°. Dried residue, 0.41 g., 8%. A rabbit weighing 660 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 730 g. Uterus normal. The tubes were congested, but this was probably connected with the fact that the ovaries were cystic. Empty body, 473 g. Dried uterus, 0.0114 g. Normal, about 0.0152 g.

No. 185. 1.22 g. of "Stock II" were dissolved in 12 cc. of glacial acetic acid. This was added dropwise to a solution of 7.5 g. of sodium nitrite in 25 cc. of water. The solution was stirred for about 15 minutes, then was extracted with 2 portions of benzene of 50 cc. each, centrifuging. The combined extracts were washed with water and centrifuged. The solution was evaporated under reduced pressure at 40°. Dried residue, 0.815 g., 67%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 522 g. was injected with 1 cc. of emulsion (Arnold sterilization). After

4 days, 615 g. Uterus normal. Empty body, 415 g. Dried uterus, 0.0116 g. Normal, 0.0080-0.0120 g.

These experiments appeared to indicate the presence of an amino group. Several attempts were made to acetylate this or any hydroxyl group which the compound might contain, but without success.

Acetyl Chloride.—No. 186. 0.69 g. of "Stock II" were dissolved in 5.25 g. of acetyl chloride and the solution was permitted to stand at room temperature over night. The next day an excess of absolute alcohol was added with cooling. The solution was evaporated under reduced pressure at 40°. The residue gave off a strong odor of hydrochloric acid. More absolute alcohol was added and the solution re-evaporated. The dried residue was odorless. It was a brown, not very viscous liquid weighing 0.80 g. A rabbit weighing 501 g. was injected with 1 cc. of 2.5% emulsion (Arnold sterilization). After 4 days, 549 g. Uterus somewhat congested and much enlarged. Empty body, 380 g. Dried uterus, 0.0576 g. Normal, 0.0080-0.0120 g.

Acetic Anhydride.—No. 187. 1.285 g. of "Stock II" were boiled gently for 2½ hours with 25 cc. of acetic anhydride and 5 g. of fused sodium acetate. After adding water, the solution was extracted twice with benzene. The extract was washed with water once and evaporated under reduced pressure at 40°. Dried residue, 1.32 g. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 660 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 672 g. A residue of unabsorbed injected material was found. The uterus was enlarged, but only slightly congested in one horn only. Empty body, 422 g. Dried uterus, 0.0479 g. Normal, 0.0080-0.0120 g.

From the fact that the portion of "Stock II" soluble in 60% alcohol gave in less than two minutes a deep crimson color with fuchsin aldehyde reagent, it was suspected that the substance might contain either an aldehyde or a ketone group. Numerous experiments were made to test this point.

Sodium Bisulfite.—No. 188. 1.20 g. of "Stock II" were dissolved in ether, the total volume being 12½ cc. An equal volume of filtered saturated aqueous sodium bisulfite solution was added and the mixture was well shaken. The aqueous layer became turbid, probably due to the separation of bisulfite crystals. After centrifuging, the ether layer, which after a time gave a heavy white precipitate, was evaporated under reduced

pressure at 40°. Dried residue, 1.32 g. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 370 g. was injected with 1 cc. of the emulsion (Arnold sterilization). After 4 days, 373 g. Uterus enlarged, but only slightly congested. Empty body, 252 g. Dried uterus, 0.0362 g. Normal, 0.0070-0.0120 g.

Phenylhydrazine.—No. 189. 1.13 g. of "Stock II" were dissolved in 11 cc. of glacial acetic acid. Two cc. of phenylhydrazine were added and the mixture was shaken well. The next day the red liquid was still clear. It was shaken with 100 cc. of water and the emulsion extracted with 2 portions of benzene of 100 cc. each. The combined extracts were shaken with about 10 cc. of concentrated hydrochloric acid. Seventy cc. of water were added and the mixture was shaken again. The aqueous layer was drawn off and extracted with 100 cc. of benzene, the extract being combined with the former ones. These were washed once with distilled water. The centrifuge was used in the last two separations. The benzene solution, still having the deep red color formerly observed, was evaporated under reduced pressure at 40°. Dried residue, 0.69 g., 60%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 797 g. was injected with 1 cc. of the emulsion (Arnold sterilization). After 4 days, 823 g. Uterus congested and enlarged. Empty body, 582 g. Dried uterus, 0.0476 g. Normal, 0.0110-about 0.0352 g.

Hydrocyanic Acid.—No. 190. 1.79 g. of "Stock II" were dissolved in 25 cc. of ether and the solution was poured into a flask containing 10 g. of 94.6% potassium cyanide dissolved in 25 cc. of water. The flask was cooled in an ice-water bath while 50 cc. of hydrochloric acid (Sp. gr., 1.19) were added dropwise. Sodium hydroxide solution was then added until the mixture was alkaline to phenolphthalein, then glacial acetic acid until it was just acid. The mixture was extracted with two portions of benzene, 50 cc. each. After centrifuging, the extracts were shaken with some water and again centrifuged. About one-sixth was lost by the breaking of a tube. The opalescent supernatant liquid was evaporated under reduced pressure at 40°. Dried residue, 1.00 g., 56%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 530 g. was injected with 1 cc. of the emulsion (autoclaved). After 4 days, 715 g. Uterus congested and much enlarged. Empty body, 503 g. Dried uterus, 0.0478 g. Normal, 0.0110-about 0.0205 g.

Hydroxylamine.—No. 191. 1.75 g. of "Stock II" were shaken with 50 cc. of alcohol. One g. of hydroxylamine hydrochloride and 2 g. of

sodium acetate were added. The mixture was heated on the steam bath with reflux condenser for two hours and then evaporated under reduced pressure at 40°. After shaking the residue with 25 cc. each of benzene and water, the emulsion was centrifuged. The flask was washed with 10 cc. of benzene and this also was centrifuged. The insoluble residue was again extracted similarly with more benzene. The extracts were shaken with water and centrifuged. The benzene solution was then evaporated under reduced pressure at 40°. The dried residue was crystalline and weighed 1.16 g., 66%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 530 g. was injected with 1 cc. of the emulsion (autoclaved). After 4 days, 588 g. The uterus was deeply congested and more enlarged than any previously noted. Empty body, 425 g. Dried uterus, 0.1378 g. Normal, 0.0080-0.0120 g. Another rabbit weighing 385 g. was injected with 1 cc. of emulsion. After 4 days, 446 g. No residue of unabsorbed injected material was found. Uterus congested and enlarged. Empty body, 265 g. Dried uterus, 0.0312 g. Normal, 0.0070-0.0120 g. Another rabbit weighing 735 g. was injected with 1 cc. of the emulsion. The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 860 g. A residue of unabsorbed injected material was found. Uterus deeply congested and much enlarged. Empty body, 545 g. Dried uterus, 0.0827 g. Normal, 0.0110-about 0.0315 g.

Semicarbazide.—No. 192. 1.295 g. of "Stock II" were shaken with 20 cc. of 75% alcohol and the emulsion was centrifuged. The clear solution was added to a solution of 1 g. of semicarbazide hydrochloride and 1.33 g. of potassium acetate in 5 cc. of water. A precipitate formed. The addition of 30 cc. of 95% alcohol gave a clear solution. Five days later it was noticed that a crystalline precipitate had formed, which, on later investigation, appeared to be sodium chloride. The solution was filtered and evaporated under reduced pressure at 40°. Dried residue, 2.16 g. This was emulsified by shaking with 25 cc. each of water and benzene and the emulsion was centrifuged. The opalescent benzene solution was decanted, about 15% being lost by an accident. The solution was evaporated under reduced pressure at 40°. Dried residue, 0.245 g., 19%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 448 g. was injected with 1 cc. of the emulsion (autoclaved). After 4 days, 520 g. Uterus much congested and enlarged. A small residue of unabsorbed injected material was found. Empty body, 360 g. Dried uterus, 0.0677 g. Normal, 0.0070-0.0120 g.

Cadmium Chloride.—Since certain of the lipoids are precipitated by cadmium chloride, several experiments were made to see whether it would precipitate the active substance.

No. 193. 1.16 g. of "Stock II" were shaken with 12 cc. of 95% alcohol and the solution was centrifuged. To the clear solution was added 0.1 g. of powdered cadmium chloride. On shaking, a precipitate formed. The next day the solution was centrifuged. On the addition of some powdered cadmium chloride a further precipitate formed. After this had been removed by centrifuging, the clear liquid gave no further precipitate with alcoholic cadmium chloride solution. It was evaporated under reduced pressure at 40°, and the residue taken up in 20 cc. each of benzene and water. After drawing off the aqueous layer, the benzene layer was centrifuged and evaporated under reduced pressure at 40°. Dried residue, 0.335 g., 21%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 514 g. was injected with 1 cc. of the emulsion (Arnold sterilization). After 4 days, 490 g. Uterus normal. Empty body, 304 g. Dried uterus, 0.0095 g. Normal, 0.0070-0.0120 g.

No. 194. 1.635 g. of "Stock II" were shaken with 16 cc. of 95% alcohol and the solution was centrifuged. To the clear liquid was added 0.3 g. of powdered cadmium chloride; the mixture was well shaken. The next day, after centrifuging, the clear liquid gave a precipitate with alcoholic cadmium chloride solution. One-tenth g. of cadmium chloride was added and the mixture shaken. The next day, after centrifuging, there was no precipitate with alcoholic cadmium chloride solution. The clear liquid was decanted and evaporated under reduced pressure at 40°. The residue was emulsified with 20 cc. of water and the emulsion extracted with two portions of benzene, 20 cc. each. The extract was centrifuged and evaporated under reduced pressure at 40°. Dried residue, 0.415 g., 25.4%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 615 g. was injected with 1 cc. of the emulsion (Arnold sterilization). After 4 days, 645 g. Uterus enlarged and slightly congested. Empty body, 465 g. Dried uterus, 0.0340 g. Normal, 0.0120-about 0.0140 g.

It was thought that possibly the active material might have been adsorbed by the phosphatide-cadmium chloride precipitate. The test was accordingly made on the acetone-soluble portion of "Stock II."

No. 195. 1.08 g. of "Stock II" were shaken with 11 cc. of acetone and the solution was centrifuged. After evaporation under reduced pressure at 40° the residue was shaken with 11 cc. of 95% alcohol. The solu-

tion was shaken with 0.4 g. of cadmium chloride. The next day, after centrifuging, no precipitate was formed on adding alcoholic cadmium chloride solution. The clear liquid was decanted and evaporated under reduced pressure at 40°. After taking up the residue in 20 cc. of water this was extracted with two portions of benzene, 20 and 85 cc. respectively. The extracts, after being centrifuged, were evaporated under reduced pressure at 40°. Dried residue, 0.35 g., 32½%. The crystalline residue was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 735 g. was injected with 1 cc. of the emulsion (Arnold sterilization). After 4 days, 760 g. Uterus congested and enlarged. Empty body, 518 g. Dried uterus, 0.0372 g. Normal, about 0.0235 g.

Fuller's Earth.—No. 196. 1.605 g. of "Stock II" were shaken with 50 cc. of 95% alcohol and 5 g. of fuller's earth. After 2 days the solution was centrifuged. The greenish-yellow, light colored supernatant liquid was decanted and evaporated under reduced pressure at 40°. Dried residue, 0.47 g., 29%. This was emulsified to the volume which would have given a 2.5% emulsion of the original "Stock II." A rabbit weighing 695 g. was injected with 1 cc. of the emulsion (Arnold sterilization). After 4 days, 830 g. Uterus congested and enlarged. Empty body, 520 g. Dried uterus, 0.0296 g. Normal, about 0.0240 g.

No. 197. "Stock III" was tested according to the method of Noyes and Mulliken.[38] Neither nitrogen, sulfur, nor halogens were found.

H. Progress toward the Preparation of the Pure Active Substance

No. 198. 1.62 g. of "Stock III" were dissolved in 10 cc. of acetone and 1 cc. of water was added. The solution was left in the ice-box for one week, when the crystals were filtered off with suction. The material was pale yellow; the yield was 0.15 g. Heated on mercury, the material softened at 51½° and melted completely at 55½° (uncorrected). It was dissolved in a mixture of 7 cc. of acetone and 1 cc. of water and the solution was placed in the ice-box. The next day the precipitate was filtered off with suction. A yield of 0.065 g. of white crystals was obtained. These softened on mercury at about 55.8° and melted at 57.1° (corrected). When tested by the Liebermann-Burchard test the material gave only a pale emerald color, instead of the usual deep green of cholesterol. 0.060 g. were dissolved in chloroform and the solution was transferred to an

ampoule. After evaporating the chloroform, a 2.5% emulsion was prepared. A rabbit weighing 710 g. was injected with 1 cc. of the emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 763 g. A residue of unabsorbed injected material was found. Uterus congested and enlarged. Empty body, 473 g. Dried uterus, 0.0416 g. Normal, about 0.0157 g.

No. 199. It had been noticed that, after evaporating the bulk of the acetone from the acetone dialysates of "Stock II," long, beautiful, needle-shaped crystals formed quite readily. 1.61 g. of material obtained by dialysing "Stock II" first in acetone, then in 75% alcohol, were dissolved in 10 cc. of warm 85% acetone (85 cc. of Merck's acetone (water 3%) diluted to 97 cc. with water). The solution was decanted and the flask was rinsed with 6.1 cc. of the dilute acetone. The combined solutions after cooling were set in the ice-box. The crystals were filtered off with suction. Yield, 0.15 g. Heated on mercury, they softened at 123° and melted at 132°. They gave a deep green color by the Liebermann-Burchard test and consequently were probably impure cholesterol. After adding 4 drops of almond oil, the mixture was dissolved in ether, which was then evaporated on the steam bath. A rabbit weighing 725 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 760 g. A residue of unabsorbed injected material was found. Only the tip of one horn of the uterus was congested and enlarged. Empty body, 526 g. Dried uterus, 0.0143 g. Normal, about 0.0250 g.

No. 200. 2.565 g. of acetone dialysate of "Stock II" were dissolved in a warm mixture of 10 cc. of acetone and 1 cc. of water. After filtration, the solution was cooled in the ice-box. The white, feathery crystals were filtered off with suction. Heated on mercury, they softened at 135° and melted at 139° (uncorrected). On recrystallization from a mixture of 25 cc. of acetone and 1 cc. of water a yield of 0.55 g. of snow-white, matted needles was obtained. These melted on mercury at 146° (uncorrected). They gave the Liebermann-Burchard test for cholesterol and also the Golodetz test (brown-black color with 3 parts of 40% formalin and 5 parts of sulfuric acid, if free cholesterol).

No. 201. The mother liquor from the first cholesterol crystallization in the above experiment was evaporated to dryness under reduced pressure at 40°. The residue was emulsified in 27.7 cc. of 75% alcohol, and the solution was dialysed through collodion into 200 cc. of 75% alcohol at 40°. The next day the first dialysate was decanted and placed in the ice-box. Three days later the needle-shaped crystals were filtered off. Yield, 0.08 g.

The material was yellowish and greasy. Heated on mercury, it melted at 127-128° (uncorrected). It gave the Liebermann-Burchard test for cholesterol. The third and fourth dialysates in 75% alcohol were evaporated under reduced pressure at 40°. The second dialysate was added. Upon warming, a clear solution was obtained which was placed in an open beaker in the ice-box. No crystallization was obtained after 10 days' standing. The mother liquor from the second crystallization of the first dialysate was then added and the mixture evaporated under reduced pressure at 40°. Residue, 1.065 g. This was dissolved in 5 cc. of acetone. The solution was filtered and the filter washed with acetone. The solution was evaporated to dryness on the steam bath. After dissolving the residue in 5 cc. of acetone, 5 cc. of water were added and the mixture was cooled to 0° in the ice-box. Acetone was added until the solution became clear. The total volume of acetone used was 50 cc. The solution was placed in the ice-box in an open beaker. After six days a few crystals resembling cholesterol had separated, also some red liquid.

No. 202. 3.39 g. of "Stock III" were dissolved in 200 cc. of warm 62½% alcohol, and the solution was left in the ice-box over night. An orange-red liquid settled to the bottom, and numerous crystals floated through the solution. The liquid and crystals were decanted from the red residue through a filter. The crystals, after drying on a porous tile, weighed 0.445 g. They were orange-yellow, shade II., by the Milton-Bradley color standard. Heated on mercury, they melted at 44-46° (uncorrected). The red residue was treated with the warmed mother liquor, but did not dissolve. After adding 30 cc. of warm 62½% alcohol and 10 cc. of 95% alcohol, used to rinse the suction flask, etc., the mixture was placed in the ice-box over night. A second crop of crystals was obtained as before. Dried on a porous tile, they weighed 0.119 g. Their color on the Milton-Bradley standard was yellow, light broken tone. Heated on mercury, they softened at 45° and melted completely at 50°. The mother liquor and red residue were evaporated to dryness under reduced pressure at 40°. Residue, 2.835 g. This was dissolved in 10 cc. of 95% alcohol, and the solution filtered. The filter and flask were washed with four portions of 95% alcohol, 10 cc. each. To the red solution, at room temperature, 6 cc. of water were added; a slight permanent cloudiness ensued. On warming, this disappeared. The beaker was covered with a watch-glass and set in the ice-box. Four days later the crystals were filtered off; they proved to be cholesterol.

In another, similar experiment, from 1.370 g. of "Stock III'" were obtained 0.225 g. of crystals, melting on mercury at 39-44°. Their color was yellow, broken tone medium on the Milton-Bradley standard. They

were added to the first and second crops of this experiment. Total, 0.775 g. This was dissolved in 5 cc. of 95% alcohol and the solution filtered. The filter was washed with 20 cc. of 95% alcohol. After adding 5 cc. of water, the emulsion was warmed to 40°. More 95% alcohol was added until the solution became clear; 27.5 cc. were required. The solution was placed in the ice-box, but no crystallization resulted in 24 hours. It was evaporated under reduced pressure at 40°, and the residue dissolved in 15 cc. acetone. Three cc. of water were added; the solution was warmed to 40° and acetone added till the turbidity disappeared; 5 cc. were required. On placing in the ice-box, crystallization ensued quickly. The solution was filtered with suction, and the crystals dried on a porous tile. Yield, 0.174 g.; color, orange-yellow, tint III. on the Milton-Bradley standard. On mercury, they softened at 53° and melted completely at 54°. A second crop was obtained similarly from the mother liquor; it weighed 0.139 g.; heated on mercury, it softened at 48½° and melted at 50° (uncorrected). Its color was yellow, light broken tone, on the Milton-Bradley scale.

The first crop was dissolved in 10 cc. of acetone, and the solution filtered; the filter was washed with 5 cc. of acetone. To the solution was added 3 cc. of water; after heating to 50°, the addition of 4½ cc. acetone cleared the solution. It was then placed in the ice-box. The next day the crystals were filtered off with suction, and dried on a porous tile. Yield, 0.066 g. The nearly white crystals, heated on mercury, melted at 54.2-55.2° (corrected). A rabbit weighing 457 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 410 g. A residue of unabsorbed injected material was found. Uterus slightly congested, but not enlarged appreciably. Empty body, 310 g. Dried uterus, 0.0083 g. Normal, 0.0070-0.0120 g.

The mother liquor from the above crystallization was evaporated under reduced pressure at 40°. Residue, 0.125 g. A rabbit weighing 650 g. was injected with 1 cc. of 2.5% emulsion (autoclaved). The material was injected into the axilla and the puncture sealed with collodion. After 4 days, 688 g. A small residue of unabsorbed injected material was found. Uterus slightly congested and locally enlarged. Empty body, 460 g. Dried uterus, 0.0238 g. Normal, 0.0115-about 0.0135 g.

IV. SUMMARY

Details are given for the extraction of the active substance from fresh ground placentas in 55-60% alcohol, for the evaporation of the solution, and the extraction of the substance by alcohol ("Stock I") or benzene ("Stock II").

A method of physiological test is described in which the effect of a preparation upon the uterus is referred to an objective standard by drying and weighing the organ. The test, while far from strictly quantitative, can be used in such a manner that roughly quantitative conclusions can be drawn from its results.

Solubility experiments show that, in general, the substance is lipoidal in its solubility. Varying results were obtained with water and benzin, depending upon the accompanying substances in the mixture.

Partition experiments with the systems water-benzene and benzin-50% or 75% alcohol show that the substance is concentrated in the benzene and alcoholic layers respectively. With water-benzin, results varied.

The substance dialysed through thin rubber in ether, acetone, benzene, and chloroform solution, and slightly in benzin and carbon tetrachloride; through collodion in 75% and 62½% alcohol, but not in 50%.

The activity was not destroyed by heat, except in the presence of air, nor was it by the ordinary reagents for hydroxyl, carbonyl, and carboxyl groups, or by reduction. It was destroyed by oxidizing agents, bromine, acids, alkalies, and nitrous acid. The most concentrated preparation obtained gave no test for nitrogen, phosphorus, or halogens. It is therefore concluded tentatively that the substance is an unsaturated compound of ester-like nature, containing no hydroxyl, carboxyl, or carbonyl groups, and no nitrogen, phosphorus, or halogens.

BIBLIOGRAPHY

1. Halban, *Archiv f. Gynaek.*, **75,** 353 (1905).
2. Claypon and Starling, *Proc. Roy. Soc. London,* **77,** Series B, 505 (1906).
3. Frank, *Jour. Exper. Med.,* **9,** 263 (1907); *Chem. Abstr.,* **1,** 1870 (1907).
4. Biedl and Königstein, *Ztschr. f. exper. Path. u. Therap.,* **8,** 358 (1910-1911).
5. Frank and Unger, *Arch. Internal Med.,* **7,** 812 (1911).
6. Aschner and Grigoriu, *Archiv f. Gynaek.,* **94,** 766 (1911); *Chem. Abstr.* **6,** 2638 (1912).
7. Niklas, *Monatsschr. Geburtsheilk.,* **38,** *Ergänzungsheft,* 60 (1913); *Chem. Abstr.,* **8,** 738 (1914).
8. Aschner, *Archiv f. Gynaek.,* **99,** 534 (1913); *Chem. Abstr.,* **8,** 180 (1914).
9. Okintschitz, *Archiv f. Gynaek.,* **102,** 333 (1914).
10. Bucura, *Ztschr. f. Heilkunde,* **28** (New Ser. 8), *Chirurgie,* 147 (1907).
11. Adler, *Archiv f. Gynaek.,* **95,** 349 (1911-12).
12. Schickele, *Archiv f. Gynaek.,* **97,** 409 (1912).
13. Sack, *Arch. f. exper. Path. u. Pharmakol.,* **70,** 293 (1912); *Chem. Abstr.,* **7,** 1216 (1913).
14. Ott and Scott, *American Practitioner,* **48,** 568 (1914); *Proc. Soc. Exper. Biol. and Med.,* **12,** 47 (1914); *Chem. Abstr.,* **9,** 3284 (1915).
15. ———, *Ztschr. f. Geburtsh. u. Gynäk.,* **77,** 49 (1915); *Chem. Abstr.* **10,** 1056 (1916).
16. Ssoloviev, *Vrachebnaia Gazeta,* **46,** 1685 (1913); *Zentr. f. Bioch. u. Biophys.,* **16,** 907 (1913-14); *Chem Abstr.,* **10,** 1887 (1916).
17. Fellner, *Zentralbl. f. allg. Path. u. pathol. Anat.,* **23,** 673, 875 (1912).
18. Parodi, *Sperimentale,* **67,** Supp., 55 (1913).
19. Fellner, *Archiv f. Gynaek.,* **100,** 641 (1913); *Chem. Abstr.,* **8,** 1149 (1914).
20. Iscovesco, *Comp. rend. Soc. de biol.,* **73,** 104 (1912); *Compt. rend. Acad. de sc.,* **155,** 1104 (1912).
21. Iscovesco, *Comp. rend. Soc. de biol.,* **72,** 858 (1912).
22. Iscovesco, *Comp. rend. Soc. de biol.,* **75,** 393 (1913).

23. Seitz, Wintz and Fingerhut, *München. med. Wchnschr.*, **61,** 1657, 1734 (1914); *Chem. Abstr.*, **9,** 219 (1915). Brit. Pat. 10,877, July 27, 1915, Soc. anon. pour l'ind. chim. à Bâle; *Chem. Abstr.*, **11,** 185 (1917). Swed. Pat. 43,031, Sept. 5, 1917, Ges. für Chem. Ind.; *Chem. Abstr.*, **12,** 516 (1918). U. S. Pats. 1,318,698 and 9, Oct. 14, 1919; Seitz and Wintz, *Chem. Abstr.*, **14,** 95 (1920).

24. Herrmann, *Zentralbl. f. Gynäk.*, **37,** II., 1563 (1913); *Wiener klin. Wchnschr.*, **26,** 1920 (1913).

25. Herrmann, *Monatschr. f. Geburtsh. und Gynäk.*, **41,** 1 (1915).

26. Aust. Pat. 71,382, March 27, 1916, S. Fränkel and E. Herrmann; *Chem. Abstr.*, **10,** 2503 (1916).

27. Brit. Pat. 113,311, Feb. 13, 1917, Soc. pour l'ind. chim. à Bâle; *Chem. Abstr.*, **12,** 1337 (1918).

28. U. S. Pat. 1,314,321, Aug. 26, 1919, S. Fraenkel and E. Herrmann; *Chem. Abstr.*, **13,** 2736 (1919).

29. Frank and Rosenbloom, *Surg., Gynec., and Obst.*, **21,** 646 (1915).

30. Frank, *Surg., Gynec., and Obst.*, **25,** 329 (1917).

31. Lee and Frank, personal communication from O. I. Lee.

32. Cova, *Ann. di Ostet.*, **37,** II., 225 (1915).

33. Gentili and Binaghi, *Ann. di Ostet.*, **38,** I., 313 (1916).

34. Gies, *Biochem. Bull.*, **2,** 55 (1912-13).

35. Rosenbloom, *Biochem. Bull.*, **2,** 64 (1912-13).

36. Henriques, *Ztschr. angewandte Chem.*, **1895,** 721 (1895).

37. Smith and Mair, *Jour. Path. and Bacteriol.*, **16,** 131 (1911); **17,** 609 (1913).

38. Noyes and Mulliken, Laboratory Experiments on the Class Reactions and Identification of Organic Substances. Ed. 3. Easton, Pa. Chemical Publishing Co., 1915.

VITA

Paul Miller Giesy was born at Cleveland, O., Dec. 24, 1890. He prepared for college at the East High School, Columbus, O. In the Fall of 1906 he entered The Ohio State University. He was graduated in June of 1910, receiving the degree of Bachelor of Arts, and in June of 1912, receiving the degrees of Master of Arts and Bachelor of Science in Chemical Engineering. From August, 1912, to May, 1914, he was Assistant Chemist, Leather and Paper Laboratory and Division of Carbohydrate Investigations, Bureau of Chemistry, United States Department of Agriculture. From June, 1914, to February, 1915, he held a position with The Procter & Gamble Co., Ivorydale, O. From February, 1915, to June, 1916, he was a graduate student at The Ohio State University, being University Scholar during the year 1915-1916. During the year 1916-1917 he was Associate in Cancer Research and a graduate student at Columbia University. From May, 1917, to January, 1919, he served as Captain, United States Army. In February, 1919, he returned to Columbia to complete his studies for the Degree of Doctor of Philosophy and resumed his work in Cancer Research.

He was co-author with Prof. James R. Withrow in a paper on "The Electrolytic Preparation of Solid Alkali Amalgams," presented before the Division of Inorganic and Physical Chemistry, American Chemical Society, at the New Orleans meeting, April, 1915.